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COLD SPRAYED COATINGS IN BIOMEDICINE

Master's thesis

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Kylmäruiskutus on kiinteän tilan pinnoitustekniikka, joka mahdollistaa monipuolisen pinnoiterakenteen valmistamisen. Sen erityispiirteitä ovat alhainen prosessointilämpötila ja erittäin suuri partikkelinopeus, jonka vuoksi rakenne on tyypillisesti tiivis ja puhdas. Sen etuna on mahdollisuus pinnoittaa pieniä aloja yksityiskohtaisesti sekä toisaalta pinnoitteen paksuus on hyvin hallittavissa. Kylmäruiskutekniikkaan liittyen on viime vuosina julkaistu monia lääketiedettä sivuavia tutkimuksia, joista suurin osa on keskittynyt ortopedisten implanttien pinnoitukseen, fotokatalyyttisiin pinnoitteisiin sekä antibakteerisiin pinnoitteisiin. Implanteilla esiintyviä kliinisiä ongelmia ovat muun muassa implantin rikkoutuminen, löystyminen, liukeneminen ja tulehdusreaktiot. Näitä komplikaatioita voidaan merkittävästi vähentää esimerkiksi parantamalla pinnoitteen ja substraatin välistä rajapintaa, estämällä tulehdusreaktioita lääkeaineella tai antibakteerisella pinnoitteella, tai eliminoimalla substraatin ja plasman välinen kontaktipinta. Tutkimuksissa on osoitettu, että kylmäruiskupinnoitustekniikalla voidaan valmistaa muun muassa antibakteerisia pinnoitteita, alhaisen kimmomodulin pinnoitteita, sekä pinnoitteita monista sellaisista materiaaleista, joiden prosessointi korkeissa lämpötiloissa on ongelmallista, kuten magnesium, titaanioksidi, hydroksiapatiitti, ja polymeeripohjaiset substraatit.

Tässä kirjallisuuskatsauksessa selvitetään tämänhetkisen kylmäruiskututkimuksen tilannetta ja tulevaisuuden näkymiä lääketieteen kentällä. Tähän liittyen tavoitteena on tarjota selkeä kuva eri materiaaleista ja materiaaliominaisuuksista. Näin ollen työ sisältää suuren määrän lähdeviitteitä, jotka kattavat merkittävän osan lääketiedettä käsittelevistä termisen ruiskutuksen artikkeleista. Yleisesti voidaan sanoa, että kylmäruiskupinnoitus on monipuolinen tekniikka, jota voidaan käyttää soluvasteen ja proliferaation modifiointiin.

ABSTRACT

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Cold spraying is an emerging coating technology with a unique and distinctive coating characteristics. The major advantages of the coatings fabricated using this solid-state deposition technology owe to low processing temperature and extreme particle velocity, resulting in dense and high-purity coating structures with a well-defined footprint and a wide range of attainable thicknesses. It has recently been a subject for increasing number of studies on potential medical applications. The majority of the cold spray studies are focused on improving the properties of orthopaedic implants but some other medically-driven research topics such as photocatalysis are current. However, in a clinical setting, implant rupture, aseptic loosening, inflammatory reactions, and material dissolution are common challenges of not only coated but of all the implants. As to coating technologies, there are several effective ways to overcome some of these problems, e.g., enhancing the bonding at the coating-substrate interface, preventing inflammatory reactions by means of antibacterial agents, eliminating the substrate from body fluid, or potentiating controlled drug release. In the context of cold spraying, the recent advances include fabrication of antimicrobial coatings, avoidance of a detrimental stress shielding –effect, and spraying of temperature-sensitive materials, such as magnesium, titania, hydroxyapatite, polymers and composite substrates. In terms of biocompatibility, also surface charge, porosity, and surface topography are central although little attention has been invested on them.

This thesis work is a literature survey on the present status and the future prospects of the cold sprayed coatings in the field of biomedicine. Therefore, the more specific objective was to produce a comprehensive review on potential materials and properties, and for that reason, a wide range of authors have been referenced. However, it was found that cold sprayed coating displayed good adhesion whereas fatigue strength, remained low. In biomedical terms, cold spraying was found as a coating method diversely capable of modifying cell response and tissue growth.

FOREWORDS

Interest was the motive for this Master's thesis which was done at Tampere University of Technology at the Department of Materials Science. I want to express my sincere gratitude to Professor Petri Vuoristo for offering me the opportunity to be part of the coatings research group as well as my fellows for the collaboration. I am tremendously grateful to my supervisor Doctor Heli Koivuluoto for the flexibility and trust, and for the advice concerning my work.

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Jonne Ketola

CONTENTS

LIST OF SYMBOLS AND ABBREVIATIONS

ABS	Acrylonitrile butadiene styrene
Actin	Component of cytoskeleton
Ag	Silver
Al	Aluminium
ALP	Alkaline Phosphatase –enzyme
Al_2O_3	Aluminiumoxide
APS	Atmospheric plasma spraying
BCC	Body-centered cubic
Bioresorbable	Material, that is completely degraded over relatively short period of time following the implantation
BMP-7	Bone morphogenic protein
Ca	Calcium
CFR-	Carbon-fibre reinforced
CFU	Colony-Forming Unit, a single colony of bacteria seen as a one entity on agar plate
CGDS	Cold gas dynamic spraying
Co	Cobalt
Collagen	Main component of ECM
Cr	Chromium
CS	Cold spraying
Cu	Copper
ECM	Extracellular matrix
FCC	Face-center cubic

FAK	Focal adhesion kinase
Fe	Iron
FGF	Fibroblast growth factor
Fibroblast	Cell characteristic to collagenous tissue
Fibronectin	Glycoprotein in ECM
GDS	Gas dynamic spraying
HA	Hydroxyapatite
Haemopoietic tissue	Tissue wherein blood cells are formed
HDPE	High-density polyethylene
HPCS	High pressure cold spray
HVAF	High-velocity air-fuel spraying
HVOF	High-velocity oxy-fuel spraying
HVSFS	High-velocity suspension flame spraying
IGF-1	Insulin-like growth factor
Integrin	Transmembrane adhesion and receptor protein
K	Potassium
KM	Kinetic metallisation
LPCS	Low pressure cold spray
Mesenchymal cells	Multipotent stem cells that give rise to cell types such as osteoblasts, chondrocytes and adipocytes
Mesoderm	Middle one of the three primary germ layers
Mg^{2+}	Magnesium ion
Mitogenic	Capable of inducing cell nuclear proliferation
MRI	Magnetic Resonance Imaging
Mn	Mangane

Mo	Molybdenum
MTS	tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium
MTT	Tetrazolium salt 3-(4,5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide
Na	Sodium
Nb	Niobium
Ni	Nickel
O	Oxygen
Osteoblast	Cells responsible for bone formation
Osteoclast	Cells responsible for bone decomposition
Osteoinducive	Capable of induce bone growth
Osteopontin	Extracellular linking protein
PA	Polyamide
PC	Polycarbonate
PDGF	Platelet-derived growth factor
PEEK	Polyetheretherketone
PGA	Polyglutamic acid
PHB	Polyhydroxybutyrate
PLA	Polylactide
PO_4^{3-}	Phosphate ion
PSPP	paranitrophenyl phosphate
PU	Polyurethane
PVC	Polyvinyl chloride

RGD	Tripeptide composed of Arginine, Glysine, and Aspartic acid amino acids
Rh	Rhodium
RUNX2	Runt-related transcription factor 2
SBF	Simulated Body Fluid
Sn	Tin
SiC	Siliconcarbide
Ta	Tantalum
Ti	Titanium
TiO_2	Titaniumdioxide
UHMWPE	Ultra-high-molecular-weight polyethylene
V	Vanadium
VPS	Vacuum plasma spraying
XTT	Tetrazolium salt 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide
Zn	Zinc
Zr	Zirconium
ZrO_2	Zirconia
W	Tungsten
α-Ti	Alpha-titanium
β-Ti	Beeta-titanium

1. INTRODUCTION

Cold spraying is an emerging coating technology with a unique and distinctive coating characteristics, which has recently been a subject for increasing number of studies made with aspiration to explore their undiscovered applications. Medical applications, being one of the most fascinating fields under research, have stirred a variety of papers published on cold spraying. Some of the studies have concentrated mostly on incremental improvements in coating properties whereas others have been more broad-minded with a pioneering approach. A vast majority of the cold spray studies, either directly or indirectly connected to medicine, are dealing with medical devices for interior use, i.e., implantology whereas the others may possess potential for exterior use. However, in a clinical setting, the implant rupture, aseptic loosening, inflammatory reactions, and material dissolution are common challenges of not only coated but all the implants. As a coating technology cold spraying can be effectively used to overcome some of these problems, e.g., in enhancing the bonding between the coating and the substrate material, preventing inflammatory reactions by means of antibacterial agents, avoiding contact between the substrate and body fluid, or potentiating controlled drug release.

The aim of this study is to survey the present status and the future prospects of the cold sprayed coatings in the field of biomedicine. Chapters 2 and 3 are dedicated to outline the technical considerations of cold spraying as well as thermal spraying. The following chapters take a step forward to more elaborately discuss the potential this novel technology holds in context of medicine. On the basis of topical issues in biomaterials science, the final chapter sums up the central findings and presents some justifications for the becoming development. The very final section is a brief account of the test methods used in the studies involved for biomaterial.

2. THERMAL SPRAY TECHNOLOGY

Thermal Spraying comprises a group of techniques exploited in spraying molten particles. As a solid-state process, cold spraying is often excluded from thermal spray family, and rather considered as its own category though it can be seen technologically evolved from thermal spray methods. Flame spraying, electric arc spraying, high-velocity oxy-fuel spraying (HVOF), high-velocity air-fuel spraying (HVAF), atmospheric plasma spraying (APS), and vacuum plasma spraying (VPS) are the most prevalent techniques of thermal spraying. The chapter below describes and sums up the salient points with respect to these techniques.

2.1. Overview on thermal spraying

There is a mutual resemblance between different processes of thermal spraying with only slight differences between the working principles. Common to all of them, a feedstock material is rapidly heated up in a chamber and propelled through a nozzle as micron-size particles onto substrate material. The coating is formed on the surface as the molten particles solidify and bond with each other. Each technique has a slightly different spray system and particularly, the design of the spraying gun depends on the used source of energy. Nevertheless, ignoring the heat generation, the operating principles are very consistent. An operating HVOF thermal spray gun is shown in Figure 2.1.

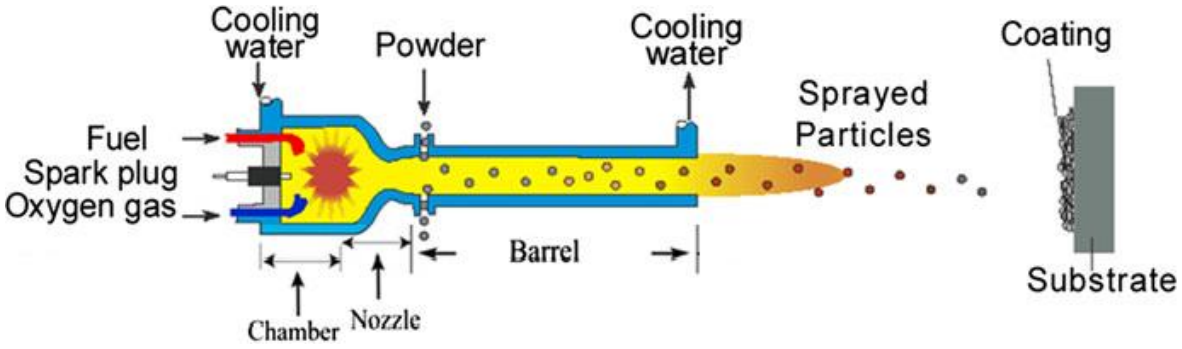


Figure 2.1. *HVOF thermal spray gun [1].*

As with cold spraying, the most essential variables between the separate techniques are the process temperature, pressure, velocity of the particles, feedstock type, and propellant gas. The variations in any of these spray parameters result in changes in coating structure. Table

2.1. presents typical values of gas temperature and particle velocity for different techniques. [2]

Table 2.1. *Some commonly applied process parameters and coating properties for thermal spray processes [3] [4].*

	Gas temp.	**Particle velocity**	**Adhesion**	**Oxide content**	**Porosity**	**Spray rate**	**Relative cost**	**Deposit thickness**
	°C	m/s	MPa	%	%	kg/h	low=1	mm
Flame	3000	40	8	10-15	10-15	2-6	1	0,1-15
Electric arc	4000	100	12	10-20	10	12	2	0,1-50
HVOF	3000	800	>70	1-5	1-2	2-4	3	0,1-2
HVAF	3000	600-1200	>70		<0.2	2-30	2	0,1-12
Plasma (APS)	12000	200-400	4-70	1-3	1-5	4-9	4	0,1-1
Plasma (VPS)	12000	400-600	>70	0	<0.5	4-9	5	0,1-1
Cold	<1000	550-1000	20-70	0	<0,5	6-8	3	0,1-2

Table 2.1 gathers up the most essential information about the characteristics of each process and the typical features of the produced coatings. Plasma spray processes employ gas temperature as high as 12000 °C whereas the uniqueness of the cold spray process is based on solid-state interactions in temperatures below 1000 °C. However, intermediate temperature range of 3000 °C to 4000 °C is applied by most of the spray technologies. Another important variable, particle velocity, reaches its maximum values, around 800 - 1000 m/s when methods such as cold spray, HVAF, or HVOF are used. Considerably lower velocities of 200 to 600 m/s are typically obtained with plasma sprays whereas the most conventional flame and electric arc sprays remain at around 100 m/s or below. Mainly due to these different combinations of gas temperature and particle velocity, the coating characteristics diverge in terms of adhesion, oxidation, porosity and attainable coating thickness as will be discussed later on in more detail.

2.1.1. Coating build-up

The structure of thermally sprayed coatings is formed as molten particle droplets collide to substrate and stack up to a coating. The resulting structure illustrated in Figure 2.2. is

dominated by consolidated droplets: splats, which are surrounded by a varying amount of oxide inclusion, pores, and unmelted particles. These structural defects have an effect on physical and chemical properties, and can be utilized in shaping the coating performance. [2]

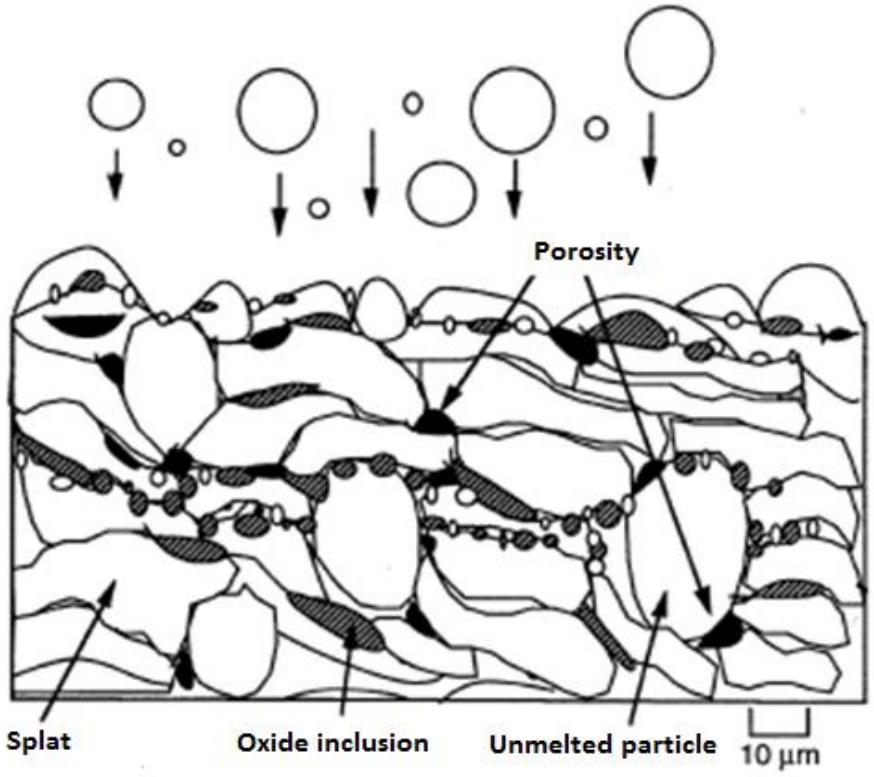


Figure 2.2. *Structure and the most common structural defects of thermal spray coatings [2].*

As mentioned earlier the oxidation is often an indication of elevated temperature that catalyses the oxidation reaction. Always increasing the temperature increases the oxidation, which can be prevented by using a shield gas. The amount of unmelted particles, in turn, decreases as the temperature elevates. Therefore, there is a trade-off between oxidation and melting of the particles and, even though this might be partially resolved by using a shield gas, it always results in oxidation to some extent. As a consequence of relatively low velocity, these structural defects cast shadows and develop pores which are usually located at the splat interfaces as illustrated in Figure 2.2. There are also other reasons for formation of highly porous structure such as post-deposition shrinkage, but both unmelted particles and oxide inclusions often are closely related to it. [2]

2.1.2. Coating properties

The coatings produced by thermal spray processes show a wide range of properties. Generally speaking, relatively low velocity and moderate temperature are characteristic attributes of flame and electric arc processes. As a consequence, an obtained coating structure is porous containing considerable quantity of impurities leading to poor erosion and corrosion resistance. Respectively, the coatings sprayed with HVOF or HVAF are usually denser than coatings sprayed with cold spray, which reflects the higher level of kinetic energy involved in these processes. The adhesion to substrate is stronger than with coatings deposited by previously mentioned processes and a wider range of materials might be sprayed due to higher temperature. The plasma processes are often preferred with ceramic materials since the high melting temperatures needs to be reached. Although it is possible to achieve very dense and pure coating structures, the plasma spraying is frequently used to produce highly porous structures beneficial in many coating applications. [5]

3. COLD SPRAY TECHNOLOGY

Cold spraying (CS), also frequently called kinetic metallisation (KM), gas dynamic spray (GDS), supersonic particle deposition (SPD), or cold gas dynamic spraying (CGDS) is one of the most novel coating technologies. It was originally discovered in the Russia in 1980's as a result of a series of experiments performed by a group of researchers led by prof. Anatolij Papyrin. Since these findings, there have been a large number of studies regarding the feasibility of cold spraying for various applications mainly during the past two decades. [6] [7] Also the focus of research has been broadened from the pure metallic coatings to cermet, ceramic, and polymeric coatings. In order to get an understanding on cold spraying as a deposition process this chapter compactly discusses the topic in more detail.

3.1. Basics on cold spraying

The basic idea of cold spraying is to deposit solid-state particles with a very high velocity to gain a coating composed of plastically deformed particles. In the cold spray process, powder particles with typical diameter of 5-50 µm are accelerated through a spray gun [8]. The acceleration is acquired with a very high velocity gas flow, which propels the particles out of a small (10-15 mm^2) nozzle with a speed required to elicit high degree of deformation upon impact with the substrate material. The applied spraying distances typically vary from 5 to 25 mm or more yielding a footprint with, depending on the nozzle, a relatively narrow diameter of around 5 mm. [8] As a result pure and dense coatings with unique properties, and most often appropriate adherence, might be obtained with wide range of thicknesses [9].

3.1.1. Deposition process

The main components of a cold spray system are low or high pressure gas supply, gas pressure regulators, gas preheaters, powder feeder, spray gun, nozzle and a control unit [9] [10]. Figure 3.1. describes more specifically the functions of a typical cold spray system including the role of computing in data acquisition and control of the spraying process.

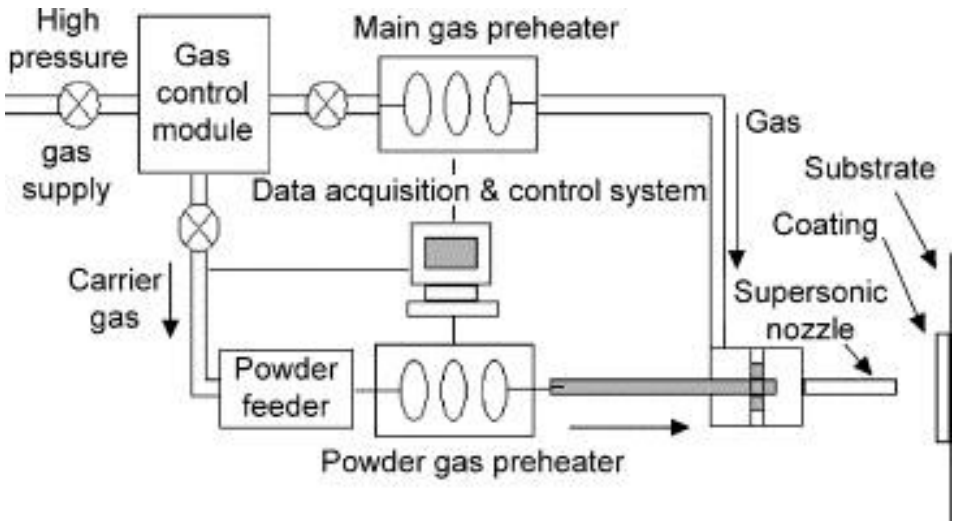


Figure 3.1. *Schematic view of a non-commercial cold spray setup [10].*

A high pressure gas, usually compressed pure N_2, pure He, mixture of N_2 and He, or dry air, is supplied into the gas preheaters through regulators that control the pressure of the propellent gas [8]. The system comprises two separate gas inlets with different designs: One inlet into the main gas preheater that is used to elevate the pressure inside the converging part of a nozzle whereas the role of the other inlet is to carry the powder within the gas stream into the nozzle. As a contradiction for what one may assume a heater is needed, but is only being used to accelerate the powder particles in a nozzle as the expanding gas rapidly gains speed. [7] Such expansion of the gas equally results from a special type of converging-diverging-shape (or de Laval-type) nozzle and typically, velocities varying from 300 to 1200 m/s are obtained [8]. However, the amount of energy brought into the process by the heater has a strong correlation with the pressure inside the nozzle and the resulting particle velocity, which highly defines the outcome. [7] An illustrative Figure 3.2. depicts a typical cold spray gun with a converging-diverging nozzle. In the converging part of the nozzle the process gas undergoes a sudden compression simultaneously with velocity gain resulting in the peak in mass flow rate at the throat of the nozzle. Further acceleration takes place in the subsequent diverging part of the nozzle, wherein the carrier gas along with the spraying particles flow with the maximum velocity out of the nozzle onto the surface of the target substrate. The highest deposition rates reached have been as high as 14 kg/h [9]

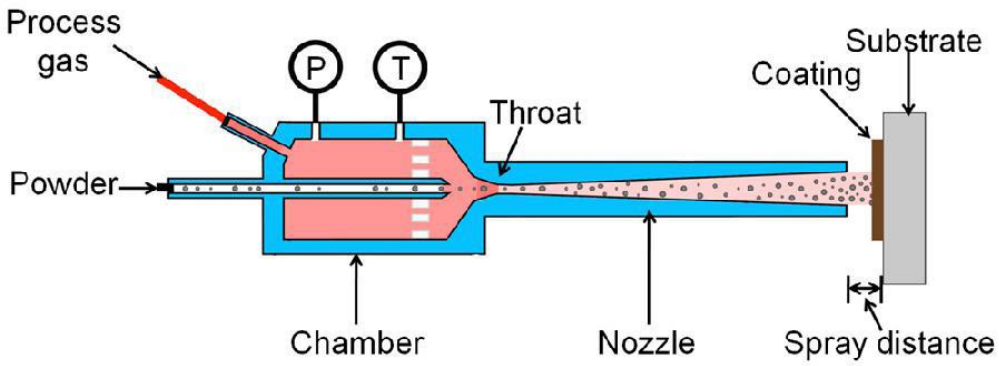


Figure 3.2. *A cold spray gun with a converging-diverging (De Laval-type) nozzle [11].*

3.1.2. Coating formation and build-up

Cold spraying is a relatively simple coating process that takes the advantage of the solid-state interactions between the deposited particles and the substrate material. During the process the sprayed particles undergo an extensive plastic deformation as they collide on the surface of the substrate. The proposed theory suggests thermal softening as the main mechanism of interaction at the site of impact as the powder particle deforms with a very high strain rate. However, though the deformation appears to contribute the bonding there is no undisputable evidence of the link between them. [9] Figure 3.3. schematically shows the stages of cold spraying process presented by Van Steenkiste et al [12].

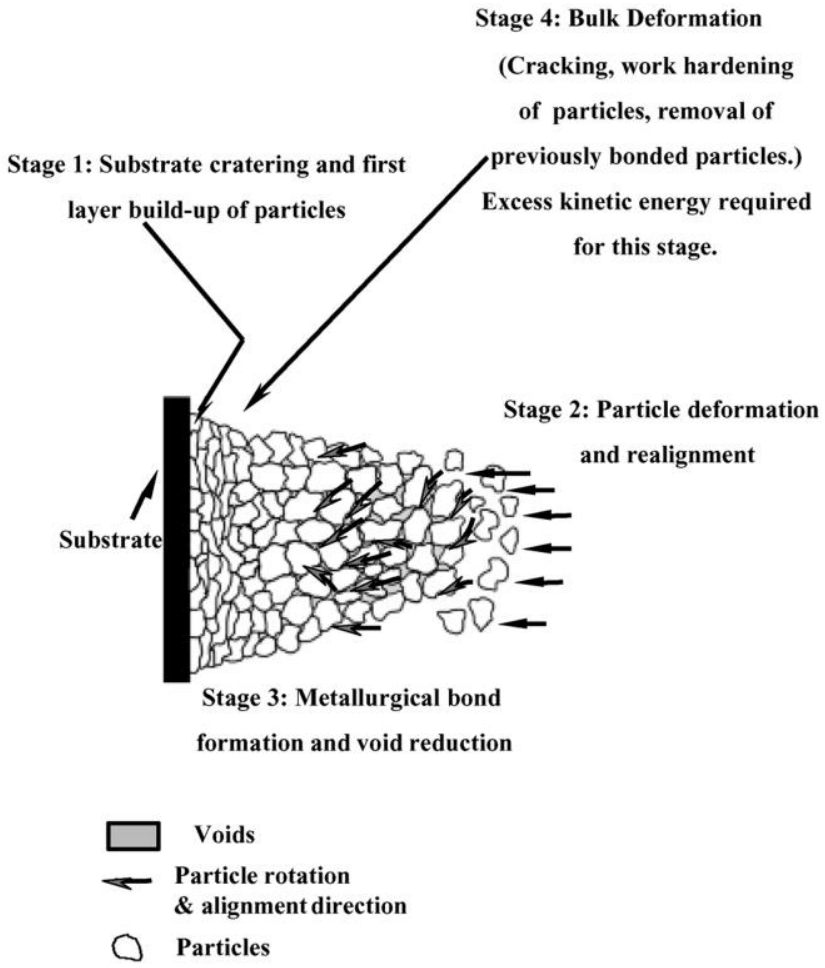


Figure 3.3. *Model for build-up process of a cold spray coating [12].*

An extensive surface deformation takes place as a consequence of the first layer of particles impinging the substrate surface. The level of deformation depends on the several factors including particle velocity, temperature, and substrate-particle interaction as well as surface preparation procedure. This initial step of the spraying process plays also an important role in removing the oxide layer and contaminants that cover the substrate and which, hence, might impede the bonding formation between the two. [12] In order to get an understanding on the incidence the Figure 3.4. plots the temperature and the stress of a Ti particle into a time scale. There is a rapid decline in the stress level prior to rise of the particle temperature. This energy shift also known as adiabatic shear instability accounts for the firm bonding that is followingly formed. [13]

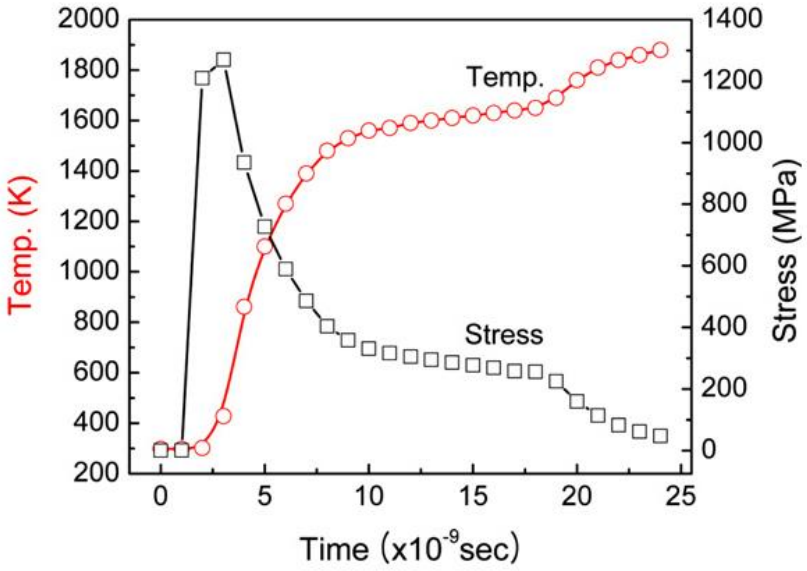


Figure 3.4. *Temporal changes in temperature and stress levels of the titanium particles during an impact on titanium substrate [13].*

The removal of the layer potentiates an appropriate bonding to form resulting from an interaction between the pure forms of the interacting materials, which are usually metals yielding, respectively, metallic bonding [14]. This is a metallurgical type of bonding where two metallic materials are closely contact with each other, but also another mechanism of bonding for metal-on-metal coatings has been established: mechanical interlocking, which is formed as an area of either coating or substrate is being extruded inside the other and mechanically trapped. Both of these mechanisms have evidenced contribution to the strength of the bonding between different coating-substrate combinations. [9] In addition to previous, a couple of other mechanisms have been proposed as well such as physical bonding, nano/micro-scale mechanical mixing, or material mixing with amorphous materials [15].

The second stage in the coating build-up model, shown in the Figure 3.3., represents the particle deformation and subsequent realignment caused by a multiple successive collisions of particles. Meanwhile, metallurgical bonds are formed between the particles at the stage three with a reduction in porosity content. During the final fourth stage the dislocation density of the bulk coating increases which has an elevating impact on the level of strength and hardness. [12] The resulting coating structure produced by cold spraying inherently exhibit a deformed, dense structure with a low porosity content and excellent adherence.

3.1.3. Process parameters

In the previous chapters, the cold spray process was outlined and the coating formation explained simplistically. The process parameters instead are the link that connects them with each other and especially kinetic energy is worth of attention because of the nature of the process. The most central parameters defining the amount of kinetic energy brought through the nozzle exit are the nozzle design, type of propellant gas, gas temperature and internal pressure whereas if the particle velocity is the subject of interest the size and morphology of the spraying particles needs to be taken into account. [16] In terms of velocity, the density and shape of the particles have an essential role. The velocity has a positive correlation with decreasing density and smaller size. [8] [9] However, the relation is affected by phenomenon called bow shock and is not therefore linear. The bow shock wave is generated in the vicinity of an object due to a rapid deceleration of the gas molecules before impingement. As a consequence, a slightly curved, stagnant zone is being constituted upon the surface of the substrate and, as opposite to acceleration, the densest particles are being affected least but the lighter particles are inflicted by more dramatic velocity loss. [8] [9] [17]

In a model created by Bae et al. [13] mechanisms of particle-substrate interactions have been divided into four separate groups according to the hardness of the coating and the substrate material. As a rule of thumb the softer counterpart undergoes a greater degree of deformation leading to thermal softening (also known as adiabatic shear instability phenomenon). The molten thicknesses based on this mathematical model varied from 11.5 nm to 53.5 nm measured from the surface. The variety of interactions occurring during impact are illustratively shown in the Figure 3.5. [13]

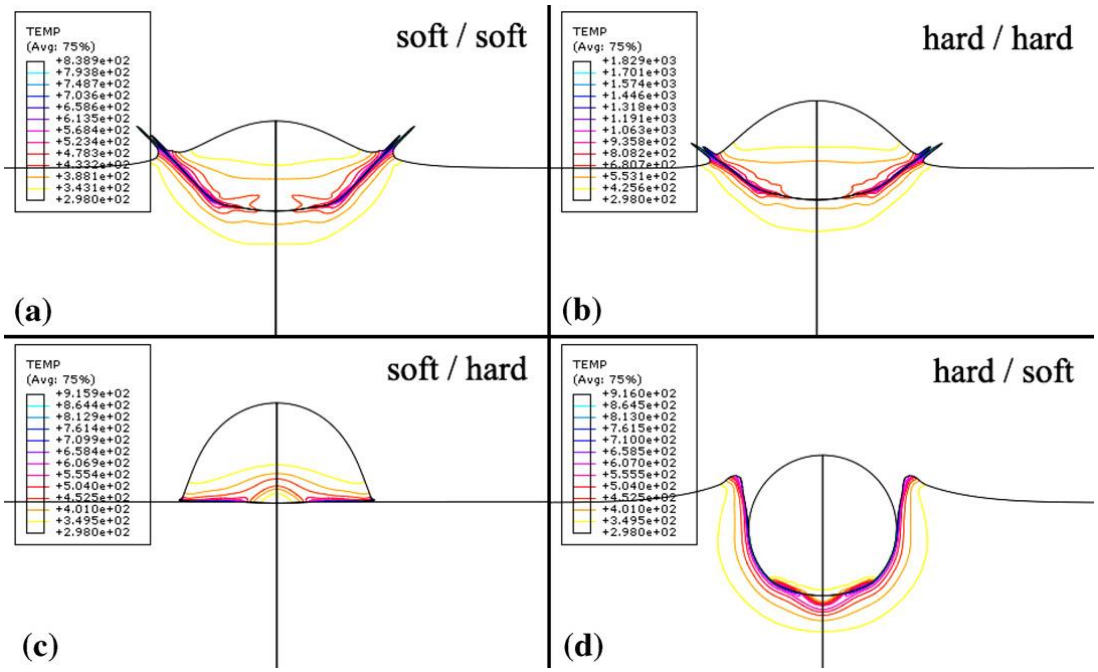


Figure 3.5. *Particle-substrate interactions with a) Al(soft) on Al(soft), b) Ti(hard) on Ti(hard), c) Al(soft) on steel(hard), d) Ti(hard) on Al(soft). Temperature zones indicate the progress of adiabatic shear instability phenomenon [13].*

As mentioned before, the particle velocity plays a key role in cold spraying. Depending on the material sprayed the velocity needs to be above the specific value of critical velocity but below the upper limit set by increasing erosion. This window of the velocities valid for cold spraying depends on the material properties and is thus material specific as the diagram in the Figure 3.6. points out. The diagram is based on the received Equation 3.1. presented by Schmidt et al. [18] for particles with 25 µm diameter and shows justified correspondence to experimentally measured values at reasonable extent [9] [16]. The equation expresses the particle velocity prior to impact

$$v_{cr} = \sqrt{\frac{A\sigma}{\rho} + Bc_p(T_m - T)} \qquad \text{(Eq. 3.1.)}$$

Where A and B are fitting constants, σ is the flow stress, ρ is the density, c_p is the heat capacity, T_m is the melting temperature, and T is the average particle temperature. Whenever the velocity remains below the critical limit the particles rebound from the surface having only abrasive effect on the substrate. For the velocities exceeding the v_{cr} value behaviour described in the previous chapter might be detected and, as long as the particle velocity remains within the window of deposition, the deposition efficiency remains steady. By convention, the critical velocity is defined as 50 % deposition efficiency, which under optimal conditions reaches its peak when nearly 100% efficiency for some materials e.g., titanium is being

obtained. [9] [16] The particles travelling with a high velocity loses its ability to attach to the surface of the substrate as the transitional surge from deposition to erosion takes place. As a consequence, the upper limit of the velocity range is set by increasing erosion. Strictly speaking, this is not the case for all of the materials and brittle ceramic particles tend to contribute the velocity-independent erosion [9]. With polymer substrates, particle embedment along with lack of plastic deformation and material mixing are usually observed leading to low shear adhesion strength of the coating. [19]

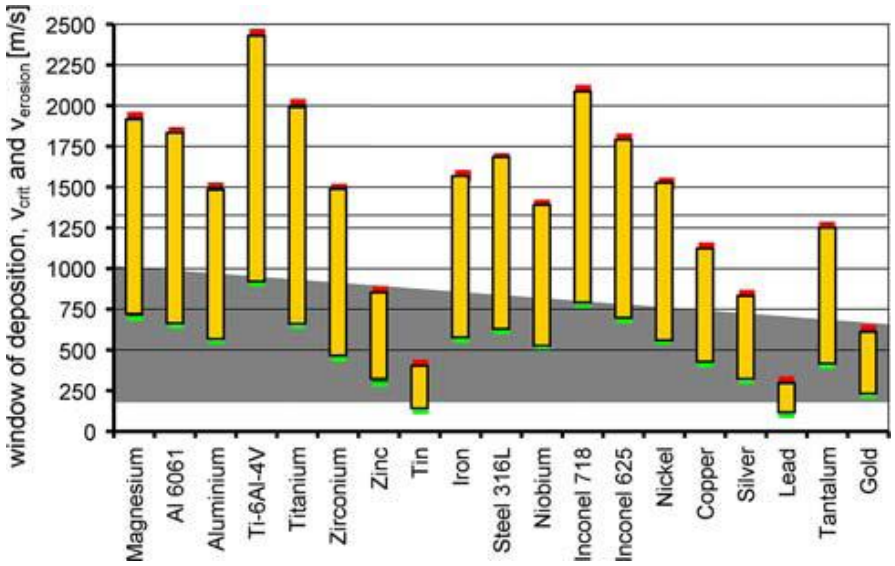


Figure 3.6. *Deposition window for a selection of metallic materials. The values are calculated for 25 µm particles with impact temperature of 20ºC. Typically commercial systems operate inside the scope of darker area. [16]*

3.1.4. Cold spray systems

The commercial cold spray systems generically fall under two classes: high pressure cold spray (HPCS) systems and low pressure cold spray (LPCS) systems [8]. Considering the former category, there are manufacturers such as Cold Gas Technology (CGT) GmbH owned by Oerlikon Metco in Germany, Impact Innovations in Germany, Plasma Giken Ltd. in Japan and Inovati Ltd. in the U.S., whereas among the low pressure systems U. S. based Centerline and Russia-based Obninsk Center for Powder Spraying are well-known manufacturers of low pressure systems [9]. The main difference between high and low pressure systems originate from dissimilar powder injection technology: low pressure systems make use of radial powder injection whereas the high pressure systems are loyal to axial injection, which accounts for gas pressures 5-10 bars with low pressure system versus pressures as high as 25-50 bars attained with high pressure system [8]. There are also differencies within high and

low pressure systems concerning the ultimate achievable gas temperature and the range of inlet pressures [9]. Some models have also taken an advantage of portability and usually, at the expense of the maximum theoretical spraying pressure, temperature, and velocity. For the reason of not compromising with the spraying parameters the stationary systems have not been replaced by portable systems.

The spraying process including particle properties is slightly divergent with low pressure system comparing to high velocity system. The particle velocity being the bottom line of the cold sprayed coatings and again, considering the fact that the coatings produced by low pressure systems often are unable to meet the requirement of critical velocity, the low pressure spraying have overcome this problem by generating a hammering effect by means of a harder feedstock powder mixed with an softer one, or using irregular shaped powders in order to promote local deformation at the particle edges. [9] Nevertheless, in terms of deposition efficiency high pressure systems are usually more favourable [8].

3.2. Materials and coating characteristics

Cold spraying has traditionally been perceived as a method viable for spraying metallic particles onto a metallic substrate, because of the deformability of the metals. These powders comprise at least the following material classes: pure metals, alloys, and metal matrix composites. [7] However, the outcome depends on the combination of particle and substrate as well as on intended target of application. In an attempt to create surfaces with improved performance there have been a number of researches accomplished with unusual selection of materials, e.g. cold sprayed ceramic hydroxyapatite (HA) onto polyetheretherketone (PEEK) polymer substrate by Lee et al. [20] exhibited strong adherence.

3.2.1. Cold spray feedstock

The selection of the spray parameters is closely associated not only with the material but purity, size distribution, and morphology of the particles. [21] As discussed in chapter 3.1.3., the critical velocity has a straight connection to particle size. This is due to high cooling rate of small particles that at first hand allows relatively large area of the particle surface to be covered with oxide scales but also impedes the formation of a proper bonding due to increased particle strength. For these reasons the adequate amount of energy required for bonding increases with decreasing particle size. [9] This mechanism appears to dominate over the fact that the net velocity of a particle increases when particle size gets smaller. Consequently, in order to produce a highly deformed and tightly bonded coating, optimised spraying conditions with a narrow particle size distribution is desired. Further, the purity

requirement is also influenced by the affinity of the material to oxidise and therefore, in order to preserve the high-purity structure, the powders that are prone to oxidation are conserved in a vacuum and sprayed under an inert propellant gas.

At the manufacturer's point of view the fabrication of particles with a certain size distribution and purity is much simpler compared to a controlled morphology or topography which might turn out to be impracticable with current techniques. Despite the related restrictions particles are commercially available in shape of sphere, hollow sphere, near-spherical, dendritic, sponge, flake, acicular, and fibrous. Choosing a particle with different morphology the surface properties of a final coating might be modified including porosity content and specific surface area. Common techniques that are utilized in particle fabrication are atomisation, precipitation, friction alloying, and vaporisation. Moreover, the particle size might be either increased by sintering, fusion, or agglomeration treatments or decreased by crushing, ball milling, or jet milling. [21]

3.2.2. Diversity of materials: powders

The majority of the research of cold sprayed coatings has been focused on metallic materials with good deformability such as copper (Cu), aluminium (Al), titanium (Ti), and nickel (Ni), but numerous other metallic materials have been successfully deposited, e.g., silver (Ag), iron (Fe), zinc (Zn), niobium (Nb), molybdenum (Mo), cobalt (Co), and their alloys [7]. There has been studies suggesting that the number of slip systems in a crystal structure is one of the factors defining the extent of plastic deformation and hence, offering an explanation for well-adhered coating structures obtained with FCC metals such as aluminium (Al), copper (Cu) and silver (Ag), whereas hexagonal metals such as alpha-titanium (α-Ti) exhibit moderate and metals such as beeta-titanium (β-Ti), iron (Fe), niobium (Nb), and chromium (Cr) with the BCC lattice lowest level of plastic deformation. [8] Therefore, challenges faced with the cold spraying when applied to metals with BCC structure such as tungsten (W), tantalum (Ta), vanadium (V), β-titanium (β -Ti) etc., are attributed to increased stress levels required to generate deformation caused by a small number of slip planes. [6] Nevertheless, the suitability of tantalum particles for cold spraying has been proven by Koivuluoto et al. [22]. Compiled list of the most common metals and alloys deposited by cold spraying is found in Figure 3.6. shown earlier.

In order to shape the functionality of a coating, the viability of cold spraying has been recognised in production of metal matrix composites providing an intimate bonding of matrix materials, typically Cu, Al, or Ni, with reinforcement particles such as aluminium oxide (Al_2O_3), silicon carbide (SiC), and titanium carbide (TiC). [7] Alike effect on bonding has been demonstrated with several other ceramic particles as well. More recently, a cold sprayed aluminium matrix has been reinforced with carbon nanotubes by Kang et al. [23] allowing control over the mechanical properties such as elastic modulus, microhardness, and wear

resistance. Moreover, a significant enhancement of overall mechanical properties was recorded. Tests made by Zhou et al. [24] with HA/Ti coatings revealed a decline in corrosion resistance proportional to hydroxyapatite content in HA/Ti composite coating. Also, a pure ceramic TiO_2 coating has been successfully deposited by Tjitra Salim et al. [25] with indications of existence of chemical bonding. As for polymer particles, there is very limited amount of information available concerning cold spraying although some speculations about the sprayability of certain thermoplastic polymers such as aromatic polyimide, aromatic polyester, polyetherketone, and fluorocarbon resin have been expressed. [21]

3.2.3. Diversity of materials: substrates

Undoubtedly as a substrate material metals and alloys hold a profound significance being the most widely explored group of substrates. Virtually any sprayable metal can be exposed to cold spraying with slight variations in ability to form bonding with impinging particles. With respect to this study there is a couple of substrate alloys worth of highlighting: ferrous alloys, cobalt-chromium alloys, and titanium alloys such as Ti-6Al-4V seen as a cold sprayed structure in Figure 3.7., wherein the higher porosity structure is formed as a result of nitrogen shield gas compared to structure produced using helium. Titanium particles, for example have been deposited onto these substrate as follows: on CoCr by Trentin et al. [26], on Ti-6Al-4V by Cizek et al. [27], and on ferrous alloys by Hussain et al. [28]

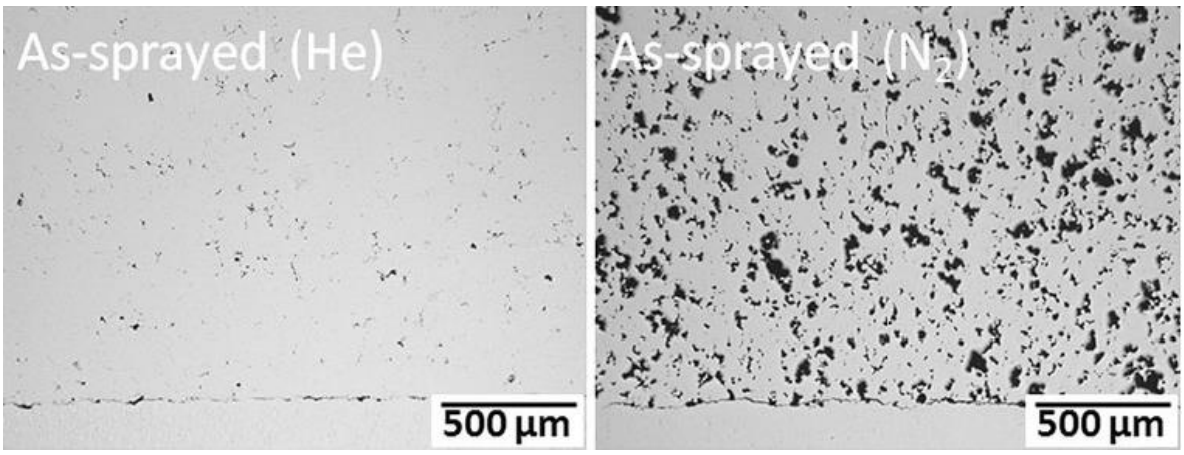


Figure 3.7. *Typical cold-sprayed structures of a traditional metallic biomedical alloy Ti-6Al-4V on substrate of the same alloy, which demonstrate the effect of helium (He) and nitrogen (N_2) shield gases on coating porosity [29].*

Recently, there have been trials demonstrating the compatibility of metallic powders sprayed onto polymer substrates. Lupoi et al. [30] sprayed Cu, Al, and Sn powders onto PC/ABS, polyamide-6, polypropylene, and polystyrene substrates. They found substrate erosion as a dominating interaction with copper particles due to necessarily high impact energy whereas

aluminium and tin with lower required impact energy were successfully deposited without considerable damage. Thereafter, similar behaviour was confirmed for copper particles sprayed onto HDPE and PU substrates by King et al. [31] with only embedment of over 50 µm in depth and erosion recorded as depicted in Figure 3.8. Instead, Ganesan et al. [19] managed to develop a thick copper coating onto PVC substrate by using large and irregular particles, also depicted in Figure 3.8. However, there is very little information available concerning e.g. the mechanical behaviour of these coatings as the research is still in its initial stage.

Polymer matrix composites is a group of materials with an advantageous ratio of strength and weight. In a study made by Robitaille et al. [32] zinc coatings deposited onto co-cured copper-embedded carbon-reinforced epoxy showed good adherence. Similarly Zhou et al. [33] reported excellent bonding of Al/Cu coating on a polymer matrix composite.

Rafaja et al. [34] studied titanium coatings on Al_2O_3 substrates and reported good adhesion which was concluded to result from mechanical interlocking consolidated by interfacial re-crystallisation of titanium and partial hetero-epitaxy. Also, titanium particles experienced plastic deformation as might have expected. Likewise, successful deposition of Al onto lead zirconate titanate was demonstrated by King et al. [35]

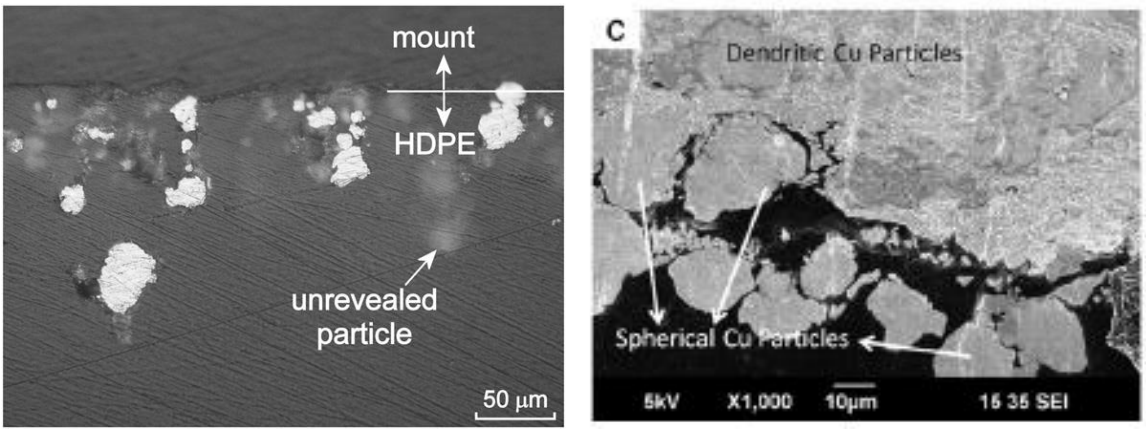


Figure 3.8. *Left-side image is a cross-sectional view of embedded copper particles deposited at 250ºC into a HDPE matrix by cold spraying [31]. Right-side image shows embedment of copper particles into PVC matrix deposited by cold spray at 200 ºC [19].*

3.2.4. Advantages and disadvantages of cold sprayed coatings

The most of the advantages of cold spraying are being attributed to either high spraying velocity or low level of thermal input. Therefore, it is convenient to divide the advantageous

coating properties according to these phenomena, which are primarily responsible for a certain properties. As noted before, cold spraying is a solid-state coating method, and due to low processing temperature a wide range of thermally vulnerable materials including polymers can be exposed to cold spray jet without considerable damage [36]. Also materials sensitive to oxygen, or possessing nano or amorphous structure might be introduced onto target surface experiencing no deleterious transformations [37]. Likewise, the final coating structure is free of spraying-induced oxides, which might have a deleterious effect on mechanical properties. The high spraying velocity induces high degree of plastic deformation resulting in strong interparticle bonding along with well-adhered coating-substrate interface. Another velocity-related reason for good bonding is shot peening-like effect that accounts for compressive residual stress of the coating but adversely the ductility of the coating is lost. However, the compressive residual stress enhances the fatigue properties, prevents micro-cracking, and enables the deposition of ultra-thick coatings without spalling. [9] [37] Despite the compressive residual stresses studies made by Cizek et al. [27] and Al-Mangour et al. [38] both failed to confirm the enhancement of fatigue properties, and in fact, found substantially weaker endurance with cold sprayed coatings under alternating loading compared to bulk specimen. The dense structure of cold sprayed coatings is manifested by good resistance to corrosion, and high thermal and electrical conductivity originating from low porosity content [37]. Furthermore, the corrosion behaviour might be improved via heat treatments inferred by Zhou et al. [39] and Al-Mangour et al. [40] One of the advantages of high velocity process is that instead of grit blasting the target surface it is roughened by rebounding particles. [9] [37] Other advantages such as high deposition efficiency and deposition rate as well as well-defined footprint depend on other factors rather than low temperature or high velocity. In spite of these advantages there is a practical weakness of cold spraying, as a line-of-sight process, for not being able to be exploited in spraying inside surfaces of hollow structures. [37] In addition, under certain conditions erosion might cause some problems especially with spraying angles 70º - 80º the substrate erodes maximally [7].

3.3. Cold spray versus other thermal spray processes

In order to appreciate the material properties of different types of sprayed coatings it is beneficial to have a comparative look on the parameters, which highly defines the resulting coating structure. Typical values of process parameters and corresponding coating properties for different spraying techniques were illustratively gathered up in a Table 2.1. The values shown here only describe a typical situation and naturally, in adjusting the spraying parameters, completely altered coating structures might be obtained.

Comparing to the thermal spray processes cold spray process has even simpler characteristic since the amount of heat energy is inadequate to melt the powder particles that are only being sprayed in solid state. This effectively prevents the formation of impurities in deposited coatings because of the absence of phase transformations including oxidation which often results in residual stresses and degradation of mechanical properties at the particle boundaries. However, high purity structures, being the most distinctive feature between cold sprayed and thermally sprayed coatings, might also be obtained with vacuum plasma spraying but in terms of cost effectiveness are unable to compete with cold spraying due to energy-consuming process as well as more expensive equipment and shield gas. Figure 3.9. below shows the applicable spraying window for each technique as a function of temperature and velocity, which underlines the extreme characteristics of cold spraying coating process. The microstructural differences are presented in Figure 3.10. with typical single splats, and another projection of the stacked splats in a final coating is shown in the Figure 3.11. with darker areas indicating the porosity.

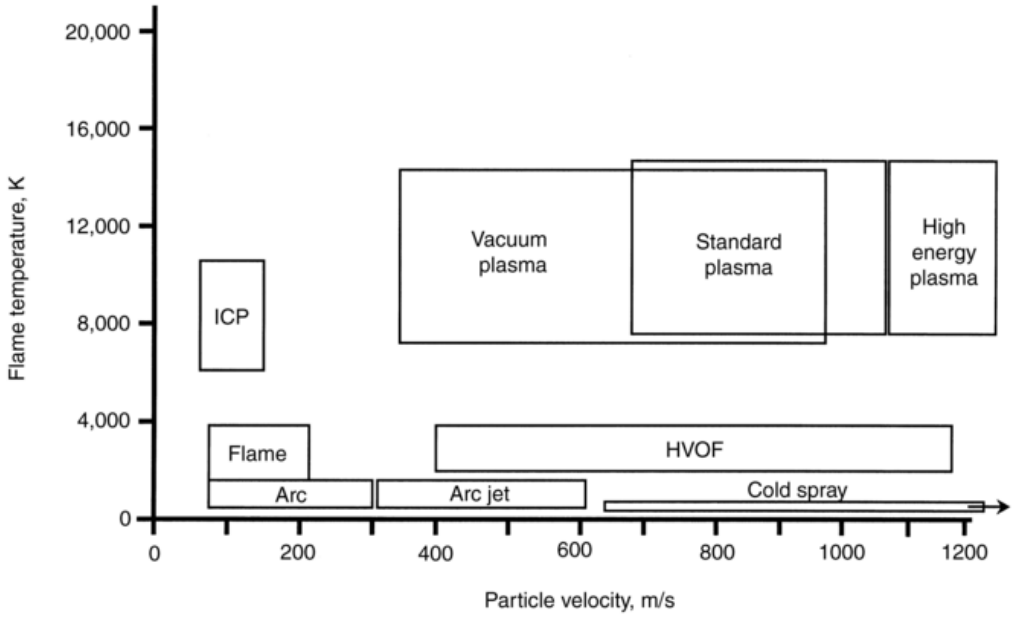


Figure 3.9. *Approximate parameter windows for some common spraying techniques [5].*

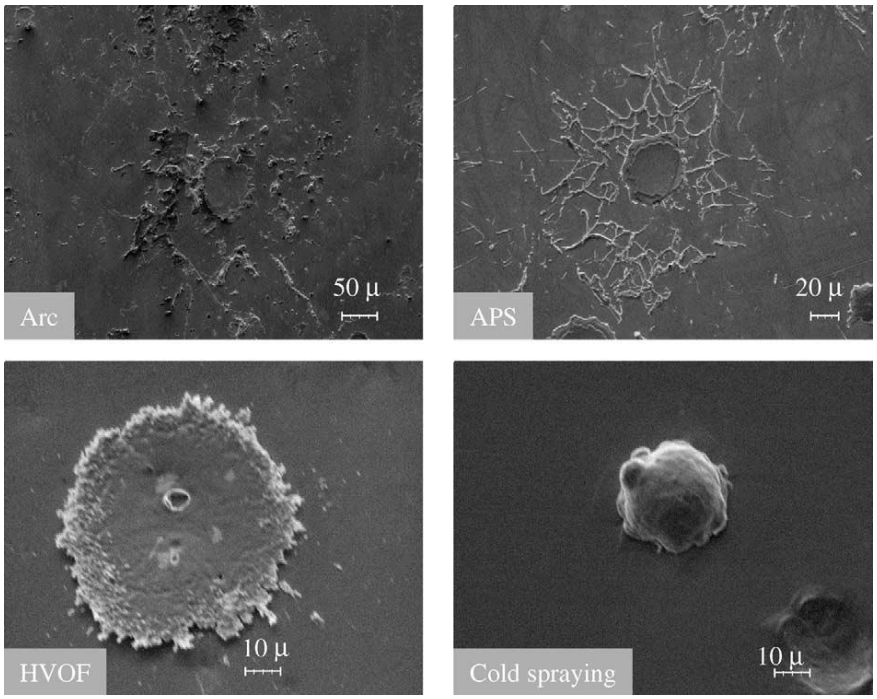


Figure 3.10. *Typical morphologies of splats (Ni-5 wt.%Al) deposited by different spraying techniques [41].*

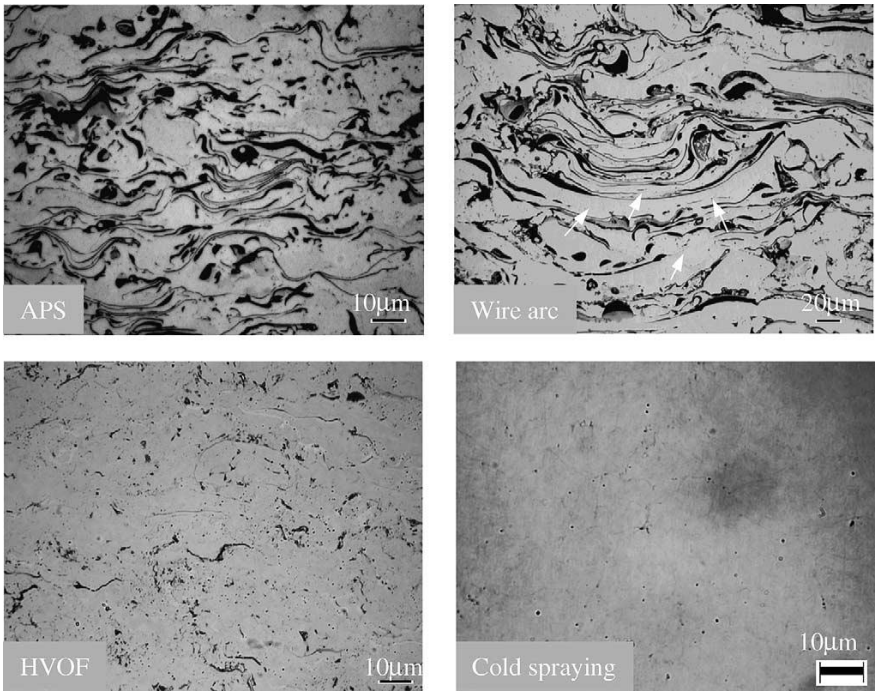


Figure 3.11. *Cross-sectional view of an "average" coating (Ni-5 wt.%Al) structure produced by different spraying methods [41].*

The major advantage of thermal spray coatings over cold sprayed ones is flexibility of material selection. In particular with plasma spraying the melting temperature of many known material is easily exceeded, which allows the process to be applied to many substrate and particle materials with no temperature- or deformability-related restrictions. However, chemically instable particles might cause problems during the process associated with preferential vaporisation and crystallisation, for example. [8] [42] In contrast, the cold spraying allows high-purity coatings to be deposited from a number of materials, and in conjunction with different material combinations, e.g., ceramics mixed with metal particles, can be effectively utilized to produce composite coatings with almost identical functionalities than coatings produced by thermal spray methods. Additionally, one of the advantages of cold spraying, though not confirmed yet, is a lack of grain growth which might presumably be useful in deposition of nanomaterials [42].

Residual stresses are quite the opposite in thermally sprayed and cold sprayed coatings. The structures created by conventional thermal methods, tensile residual stresses are developed as the coating cools down and undergoes a shrinkage whereas the corresponding coating prepared by cold spraying retain the compressive nature of the residual stresses. Low ductility values are inherent for all of the sprayed coatings in as-deposited condition but might be restored with cold sprayed coatings in heat treating because there are no phases present [42] Unexpectedly, the mechanical properties under cyclic loading has been found more degraded by cold spraying than plasma spraying regardless of compressive residual stress of cold sprayed coatings by Cizek et al. [27] Nonetheless, the cracking of the coating is usually avoided and very thick coatings might be successfully deposited. In conclusion, it is reasonable to argue for increasing importance of cold spraying as a coating method based on the reasons listed in the Table 3.1.

Table 3.1. *Some of the advantages of cold sprayed coatings.*

Accurate deposition with well-defined footprint
Wide range of coating thicknesses achievable
Dense coating structure with bulk-like properties
Very high-purity coating structure
Suitable manufacturing technology for mass production
Deposition of temperature-sensitive materials
Low thermal energy consumption

4. MATERIALS IN BIOMEDICAL APPLICATIONS

In the advent of biomimetic materials highly antibacterial and biocompatible surfaces are desired, but in terms of material properties, the substrate material plays a key role in adapting the mechanical and chemical performance to surrounding tissue. Therefore, recent development of novel composite materials with bone-like properties has resulted in increasing popularity of plastic composites at the expense of conventional metallic bone substitutes. On the other hand, biodegradability is often of a great value. Consequently, the characteristic differences of material properties need to be clarified to appreciate the ongoing evolution. A brief introduction on clinically dominant and most potential materials will be given in the present chapter along with a consideration of the viability for clinical use.

4.1. Metallic materials

Metallic biomaterials have become extensively utilised in biomedical devices for hard tissue replacements. The reason for the popularity of metals in such applications is fundamentally superior mechanical properties and highly reliable mechanical performance. However, there are some driving forces to further upgrade the performance of metallic implants. Firstly, a major concerns associated with the use of metals are the dissolution and biochemistry of the elements involved and the associated cellular interactions. Secondly, metallic biomaterials are not, generally speaking, bioactive of type and hence there is a demand for enhancing the biocompatibility. Materials such as ferrous (Fe) alloys, cobalt-chromium (Cr) alloys and titanium (Ti) alloys have become established the most prevalent metallic materials in prosthetic applications. Also, from the perspective of cold spray technology the role of metallic materials has been pronounced and is to be discussed here accordingly, albeit the other groups of materials have been recognised as potential candidates for biomedical coating applications as well. At present, metals such as zirconium (Zr), magnesium (Mg), titanium (Ti), tantalum (Ta), and niobium (Nb), are receiving attention in the biomedical context.

4.1.1. Ferrous (Fe) alloys

Austenitic stainless steel 316L has been an important alloy in prosthetic implants regardless of its risk profile. Typical problems arise from the mechanical properties well above those of natural tissues and the restricted biocompatibility with inert interactions accompanied with elevated concentrations of cytotoxic Cr- and Ni-ions. Also, some other Fe-based alloys such as Fe-Mn have been investigated as a biodegradable material showing low degradation rate.

[43] [44] To date there has been attempts to demonstrate the behaviour of cold sprayed steel including Al-Mangour et al. [38] [40].

4.1.2. Cobalt-chromium (CoCr) alloys

Co alloys e.g., Co-28Cr-6Mo, display a good resistance against wear and corrosion, which are the main advantages in comparing to stainless steel [45]. Otherwise CoCr alloys possess very similar properties to 316L. The related concerns are mainly identical to stainless steel as to biocompatibility and mechanical properties. Taking only technological aspects into consideration, the use of stainless steel 316L and CoCr alloys in the biomedical in vivo applications is expected to decrease, as these alloys are regarded as inexpensive substitutes with inferior performance for titanium alloys. [43] [44] Nonetheless, Co-20Cr-15W substrate has been exposed to cold spray deposition by Trentin et al. [26].

4.1.3. Titanium (Ti) alloys

The diverse group of Ti alloys has become the golden standard in numerous applications due to more bone-like mechanical properties comparing to conventional stainless steel and CoCr alloys, especially remarkably lower elastic modulus, high fatigue strength and favourable combination of strength/density not compromising with corrosion resistance. A major limitation of mechanical performance of Ti alloys is a relatively poor wear resistance, which accounts for its limited use in load bearing articular implants wherein abrasive wear unavoidably takes place. Among the metallic materials, Ti exhibits the most favourable cellular response when inserted in a body. In terms of popularity, Ti has been a subject of a wide range of studies at present and in the past because of the uniqueness of its properties. It is reasonable to anticipate that the Ti alloys keep increasing popularity in the field of endoprosthetic applications. [43] [46] Originally Ti-6Al-4V was widely used and was followed by Ti-6Al-7Nb and Ti-5Al-2.5Fe with lowered degree of toxicity [44]. Presently, as an attempt to eliminate the cytotoxic elements such as Al and V, multiplicity of promising alloys with elements such as Zr, Ta, Nb, Mo, Fe, etc., of which the following alloys: Ti–29Nb–13Ta–4.6Zr, Ti–30Ta and Ti–45Nb, are now considered the most favourable [47]. In economical respect, however, alloying elements such as Mn, Fe, Si and Sn are more cost-effective alternatives [48]. Additionally, clinical success of titanium containing alloys with shape-memory effect is expected to continue. However, due to postulated intolerance of Ti–Ni alloys under aggressive environment a number of novel Ti based alloys with improved performance are under investigation in order to satisfy the requirement of superelastic behaviour. [49]

Owing to appropriate biocompatibility, pure Ti and Ti alloy coatings have been under intensive biomedically-oriented research and have been introduced onto substrates by both thermal and cold spray techniques. To date the conventional biomedical alloy of Ti-6Al-4V

is used almost as a standard substrate whenever biomedical coatings are experimented but e.g., pure Ti is being utilised as well. Pure Ti powders were propelled with cold spray –setup onto Ti-6Al-4V by Cizek et al. [27] and Price et al. [50], and onto a steel substrate by Kim et al. [11]. Ti-6Al-4V powder was cold sprayed onto Ti-6Al-4V by Vo et al. [29]. Likewise, Ti-6Al-4V alloy has been the substrate for HVOF-sprayed hydroxyapatite (HA) by Li et al. [51] and TiO_2 by Lima et al. [52]. Pure Ti substrates were made use of with plasma spraying of HA by Laonapakul et al. [53] [54]. Mixing Ti with HA has been the approach to obtain plasma-sprayed composite coatings by Zhou et al. [55] and Gu et al. [56].

4.1.4. Zirconium (Zr) alloys

Zirconium is an inert material with characteristics analogous with Ti. The biomedical potential of Zr alloys is mainly based on minimal magnetic susceptibility i.e. there is very little distortion induced by Zr when an emerging imaging modality, magnetic resonance imaging (MRI), is applied. However, the use of Zr alloys is presently very rare but promising area. [44] A limited amount of studies have been published on Zr coatings produced by any of thermal spray methods. However, one such study was conducted using gas tunnel type plasma spray by Yugeswaran et al. [57].

4.1.5. Magnesium (Mg) alloys

A rapid progress has recently taken place in the field of biodegradable metallic implants thanks to rediscovery of the readily dissolvable Mg alloys [58]. In comparison with CoCr or Ti alloys the mechanical properties of Mg alloys show better match with those of natural bone. The overall biocompatibility of Mg alloys is characterised by the emergence of Mg corrosion products, mainly Mg^{2+}, and, as these ions are inherently present in vivo having various metabolic functions including stimulative effect on bone reproduction, no direct interference in surrounding tissue is caused by Mg cations. Instead, some severe symptoms of sympathetic nervous system are associated with an elevated Mg levels in serum. Another considerable downside of a rapid degradation of Mg is hydrogen (H_2) evolution manifested by endogenous cavities filled with gas. [59] Therefore, a controllability of the biodegradation of Mg alloys has been a subject of studies and particularly, the role of coatings in adjusting degradation rate to preserve the mechanical integrity of a Mg substrate has been under investigations [60] [61] [62]. Commercially available Mg alloys, which often contain cytotoxic elements, e.g., Al, has been neglected and now the focus is on the development of alloys such as Mg-Ca, Mg-Zn, Mg-Nd, Mg-Y, and Mg-Mn in which the release of the alloying elements is involved in bone tissue regeneration [63]. Also, being aware of thermal sensitivity of Mg, these alloys are of particular interest from the point of view of cold spraying. Recent scientific advances have been reported on cold spraying of Mg -alloy

substrates by Noorakma et al. [62] and Wang et al. [64] whereas Mg coating was obtained by Suo et al. [65] [66].

4.1.6. Tantalum (Ta) alloys

High biocompatibility of Ta comparable to that of Ti has been documented by several authors including Findlay et al. [67], Tang et al. [68], Wang et al. [69], and Kaivosoja et al. [70], which in conjunction with excellent chemical stability accounts for prevalent use of Ta as a porous scaffolds in order to promote tissue in-growth. [44] Based on comparative study on cellular response of human mesenchymal stem cells on metal surfaces Stiehler et al. [71] proposed Ta over Ti for biomedical implants. However, Ta coatings prepared by cold spray methods have rarely been under investigation: cold sprayed Ta coatings were studied by Koivuluoto et al. [22] and Bolelli et al. [72], and produced by vacuum plasma spraying by Tang et al. [68].

4.1.7. Niobium (Nb) alloys

There is a resemblance of physical and chemical properties between Nb and Ta, but to our knowledge clinical use of Nb alloys has not been reported. However, the potential of Nb–2Zr and Nb–28Ta–3.5 W–1.3Zr has recently been recognised and favourable performance in preclinical investigations has been confirmed along with magnetic properties that enable an inspection with MRI. [44]

4.2. Ceramic materials

Unlike with metallic and polymeric biomaterials, very little data has been documented about sprayed coatings applied to ceramic substrates, although they are of great significance in the applications such as dental replacements and bone substitutes. Nevertheless, ceramic powders are widely deposited by thermal spraying in order to enhance biocompatibility or wear resistance. Unfortunately, brittle behaviour displayed by covalently bonded ceramic materials is a substantial limiting factor of otherwise biomimetic ceramics structurally analogous to bone.

4.2.1. Titania (TiO_2)

An exceptional combination of bioactivity and photocatalytic properties has been the motive for active research on the field of TiO_2 coatings [73]. As a semiconductor TiO_2 degrades organic substances when it is exposed to electromagnetic radiation [74]. The great potential of cold spray method in fabricating such coatings relies on the fact that low temperature

process is required to preserve photocatalytically active anatase structure. Also, a passive layer of TiO_2 is inherently present at the surface of pure Ti. Progress in the field of thermal and cold spray deposition of TiO_2 is found in the referred papers: cold sprayed TiO_2 by Tjitra Salim et al. [25] [75], and Kliemann et al. [76]; HVOF sprayed TiO_2 by Lima et al. [52], Melero et al. [77], Ibrahim et al. [78], Gaona et al. [79], and Legoux et al. [80].

4.2.2. Alumina (Al_2O_3) and Zirconia (ZrO_2)

As to merits, Al_2O_3 and ZrO_2 have much in common: high resistance to corrosion and abrasive wearing, low friction, and mechanical properties of orders of magnitude higher than those of HA. Hence, load bearing dental and orthopaedic implants have taken the advantage of these beneficial properties. In contrast the evidence of complications connected to Al_2O_3 and ZrO_2 suggests stress shielding. [81] [82] Plasma spray technology has been applied by Wang et al. [83] to produce ZrO_2 coatings.

4.2.3. Hydroxyapatite (HA)

A plenty of calcium phosphate phases exist such as hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$, tricalcium phosphate $Ca_3(PO_4)_2$ and tetracalcium phosphate $Ca_4P_2O_9$ which are stable in slightly different conditions [81]. HA being a structural analogy of the main constituent of natural bone, has become a state-of-the-art biomaterial but, due to mechanically vulnerable structure, is not valid for applications wherein an ability to withstand mechanical loading is required. Therefore, calcium (Ca) compounds are now used to improve biocompatibility in a variety of devices that are directly in contact with bone tissues. Consequently, endless list of insightful research papers have been written to elucidate the characteristics of thermally, and specifically plasma sprayed hydroxyapatite coatings, such as Laonapakul et al. [53], Gu et al. [56], Legoux et al. [80], Gledhill et al. [84] and some thorough reviews have been published such as Heimann [85]. Among thermal spray coatings [86], suspension spray systems have been of a special interest: Bolelli et al. [87] studied the high-velocity suspension spraying of HA. Moreover, properties of suspension plasma sprayed HA have been revealed by Podlesak et al. [88], Latka et al. [89], Kozerski et al. [90] and Bolelli et al. [91]. Tests validating the deposition of HA coatings by cold spraying have been by Noorakma et al. [62], Lee et al. [20] and with HA composite by Liu et al. [92]

4.2.4. Bioactive glass

Bioactive glass is a broadly defined group of glasses with different compositions that allow it to establish a steady bonding with the living tissue. Irrespective of excellent biocompatibility the use of bioactive glass is restricted to small components by its brittle nature. [82] Therefore bioactive glass might be a beneficial coating material and, within the past few years high-velocity suspension sprayed bioactive glass coatings of composition

$K_2O–CaO–P_2O_5–SiO_2$ have been under investigation by Bolelli et al. [87] [93] [94]. Slightly different composition was found from plasma sprayed glass of Monsalve et al. [95]. $MgO–CaO–SiO_2–P_2O_5$ system has been prepared by plasma spraying by Cannillo et al. [96] [97] and $Na_2O–K_2O–CaO–MgO–P_2O_5–SiO_2$ system by suspension plasma spraying by Cattini et al. [98] Recently, some other interesting Ca-based glass-ceramics, such as $Ca_2ZnSi_2O_7$ and $CaTiSiO_5$, were prepared by plasma spray by Wang et al. [99] and Yu et al. [100]. In contrast, no source is available concerning cold spraying of bioactive glass.

4.3. Polymers and composites

The idea of substituting hard-tissues with some polymeric implants is supported by the resemblance of the mechanical properties with natural bone. However, in terms of thermal spraying, polymers are perceived as a marginal group of materials because of the challenges in optimising the spray parameters in order to avoid detrimental transformations triggered by elevated temperature. Another limiting factor is associated with an interaction between the substrate and the impinging particles: When hard particles are applied in considerably softer polymer substrate, substrate erosion and particle embedment are usually observed. [31] [33] Regardless of these limitations, the use of cold spray technology is not solely confined to metals or ceramics anymore, but the potential of spraying polymers under subcritical temperature has attracted wide attention.

4.3.1. Polyetheretherketone (PEEK)

An established practice of implanting biomaterials made of PEEK has been validated by its sufficient chemical stability, good mechanical properties and the fact that PEEK provides a reduction in MRI artefacts in comparing to metallic materials. Encouraging clinical results has been reached in the field of intervertebral disk replacements, but indications for manifold other applications have been proposed. [101] [102] [103] [104] Furthermore, PEEK matrix reinforced with carbon-fibre has been tested as an attempt to improve the mechanical performance. [104] However, due to inert behaviour of the PEEK an intimate bonding and stable fixation with host bone tissue are rarely obtained. Some achievements and preliminary results in the field of cold spray technology applied to PEEK substrate are found in the following scientific papers: HA-coated PEEK by Lee et al. [20], Al/Cu-coated PEEK by Zhou et al. [33], Ti-coated PEEK by Gardon et al. [105]. Carbon-fibre reinforced (CFR) PEEK was coated with Ti by plasma spraying by Devine et al. [106] Further studies concerning plasma sprayed HA coatings on PEEK has been conducted by Wu et al. [107] and Beuvais et al. [108].

4.3.2. Polyamide (PA)

Polyamide (PA), also known as nylon, is a material with a widespread use for medical devices. Two types of polyamides, PA6 and PA66, are commonly exploited and reinforced with glass fibres. [109] The primary drawback exhibited by PA is hydrolysis resulting from high absorbance for water and possible downgrading of mechanical properties. [110] Unfortunately, such corruption becomes apparent also in the presence of alcohols or ketones [109]. Innovative metallic coatings prepared by cold spray technique on PA was experimented by Lupoi et al. [30], whereas the viability of PA for cold spraying was modelled by Grujicic et al. [111]. Plasma-sprayed HA coating and HVOF-sprayed nano-TiO_2 coating was prepared on carbon-fibre reinforced PA12 by Legoux et al. [80] Similarly, CFR-PA12 was plasma sprayed with hydroxyapatite by Auclair-Daigle et al. [112] and Hacking et al. [113].

4.3.3. Ultra-high-molecular-weight polyethylene (UHMWPE)

Ultra-high-molecular-weight polyethylene (UHMWPE) is predominantly the biomaterial of choice in joint surfaces of modern articular implants owing to its excellent tribological and mechanical properties [114]. On the other hand there is a considerable disadvantage associated with UHMWPE of wear rate remarkably higher than with most metals or ceramics [81]. Additionally, the degradation of mechanical properties induced by oxidative free radicals occasionally takes place and is the major reason for UHMWPE-related complications. [114] An investigation on cold spraying of PE was performed by King et al. [31].

4.3.4. Biodegradable polymers

There are plenty of interesting polymers such as polylactide (PLA), polyglutamic acid (PGA), polyhydroxybutyrate (PHB), etc., regarded as biodegradable due to relatively prompt oxidative processes as well as hydrolysis which, concurrently lead to dissolution of the biomaterial. However, the problems are frequently encountered concerning the controllability of degradation and the adverse effects of degradation products. [63] [110] To the best of our knowledge, no literature on cold spraying of these polymers exists, but a pioneering approach has been taken with conventional flame spray equipment by Chebbi et al. [115] [116].

5. REQUIREMENTS FOR MEDICAL IMPLANTS

Presently the success of many medical treatments relies on the use of materials that are fabricated exterior to human body and are intimately brought into contact with living tissue, called biomaterials. For convenience, the biomaterials dedicated to endogenous use are mainly discussed here rather than those used externally. A listing of some endogenous implant applications are expressed in Figure 5.1.

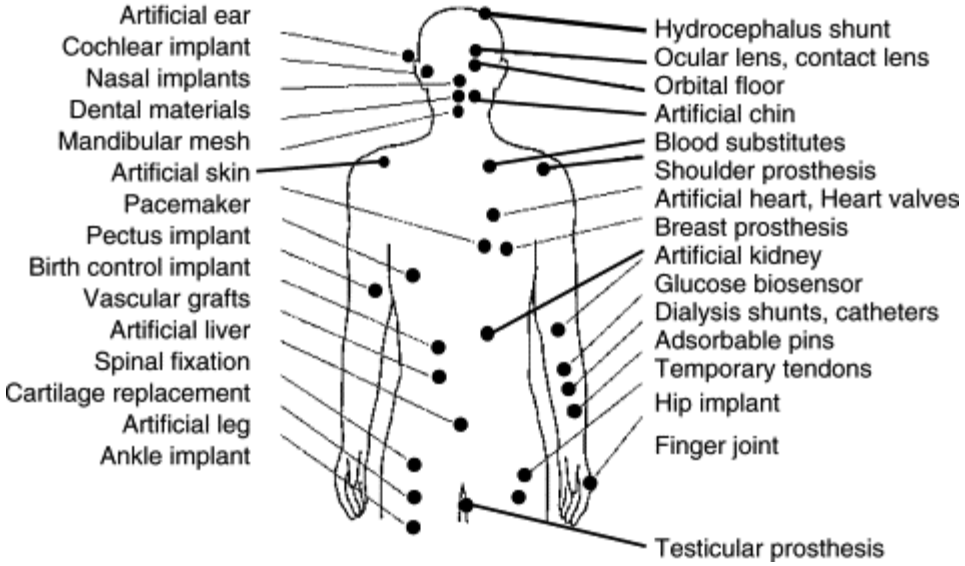


Figure 5.1. *Overview of the endogenous applications for biomaterials [117].*

At the scientific point of view the understanding of material requirements for biomedical devices and more specifically for biomedical coatings produced by cold spray technology is based on current theories of biocompatibility. The field is enormous covering a number of diverse subcategories, such as, metabolism of thousands of cell types including inflammative bacteria, protein adsorption, and extra-cellular matrix formation. The importance of biocompatibility is underpinned whenever coatings are addressed as direct contact between the coating and a living tissue is established. In many occasions the consequences of inappropriate coating performance are life-threatening leading to revision surgeries with increased risk of further complications. The reasons for deterioration of implant performance and subsequent revision surgery might be divided roughly into two major classes: deterioration of mechano-functional properties and inadequate biocompatibility. Mechano-functional properties are defined here as the ability of implant to maintain its mechanical performance whereas biocompatibility is perceived as the type and extent of either local or

systemic response generated by an implant material in living organism i.e. in vivo. However, the material properties such as hardness need to be taken into consideration with both occasions. Also important phenomena of corrosion and wear have a strong influence on both mechanical performance and biological response which accounts for the fact that these are elaborated separately in chapter 5.3. Gepreel et al. [48] outlined the causes for implant failure schematically as presented in Figure 5.2.

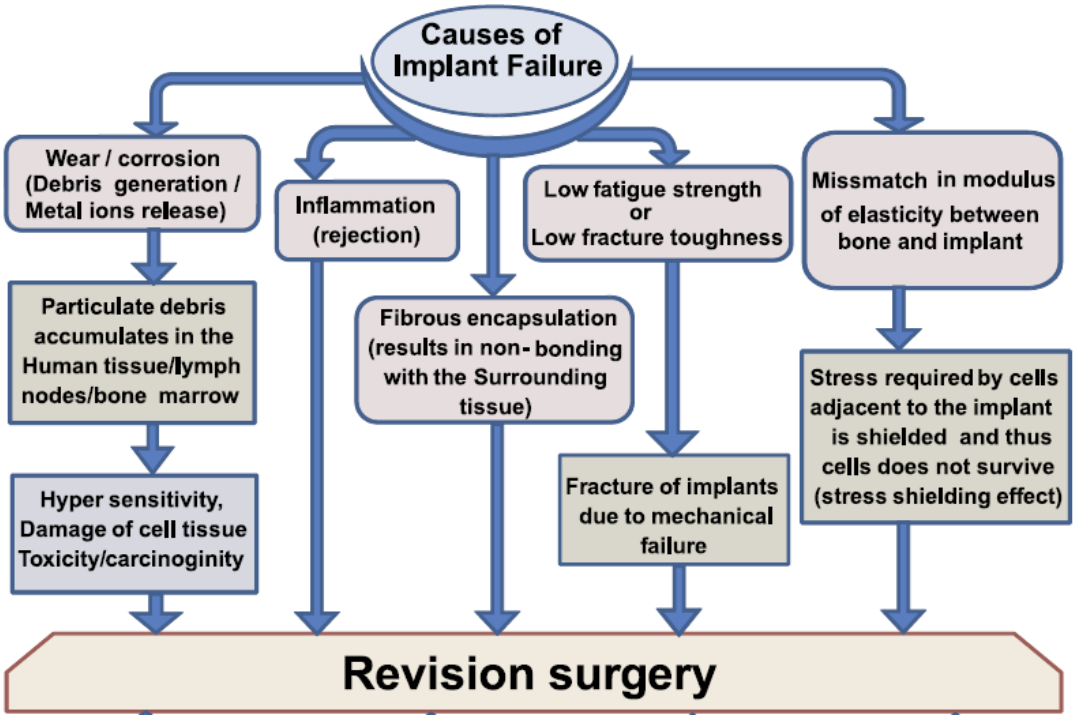


Figure 5.2. *Five basic mechanisms for implant failure. Modified from [48].*

5.1. Human body as an environment

The extreme complexity of a human body as an environment makes it very challenging target of engineering. From this perspective, at the centre of the complexity are the biochemical reactions determined by a numerous tissue-specific factors. Hence, it is of great importance to consider these factors in detail to get an appreciation of the tissue response and behaviour of the materials in vivo as the entire concept of biocompatibility relies on interactions between specialised cells. There are over 5000 different types of cells, differentiated at the early stage, that collectively constitute tissues that are dedicated to serve a specific function. The regeneration, differentiation, growth and apoptosis of these tissues are regulated by numerous endogenous substances including hormones, cytokines, and growth factors, which are also considered attractive alternatives to enhance the integration of tissue-implant

interface with novel implant materials. According to a categorisation widely acknowledged in medicine, tissues fall into four basic categories: epithelial, muscle, nerve, and connective tissue, of which connective tissue provides functional and structural support to tissues of other three types. Characteristic to this type of tissue, the cells are typically scattered and reside within abundant extracellular material produced by cells, called extracellular matrix (ECM). In embryological terms, connective tissue origins from mesoderm layer differentiated to mesenchymal cells. Later on these cells differentiate into a range of connective tissue cells including loose and dense connective tissues, bone, cartilage, adipose tissue, blood, haemopoietic tissue, and lymphatic tissue. Such tissues are responsible for the contact at the biomaterial-tissue interface, and therefore, mesenchymal stem cells have widespread use in *in vitro* –testing of the medical biomaterials. [118]

Most often the biochemical reactions may only effectively occur in certain chemical conditions, i.e., within a specific range of acidity. For this reason the chemical stability of human system is mainly maintained by HCO_3^--buffering in the blood at constant pH level of around 7,4 for arterial blood. However, there are slight systemic variations in the chemical state of the extracellular space of an individual depending on, e.g., physical activity and nutrition, but considering the implant surfaces the local instabilities in acid-base equilibrium resulting from oxidative agents produced by cells for example, has greater relevance. [119] Good examples of this are the lysosomal degradative agents of macrophages, which have acidity of pH 4 and are frequently found in high concentrations at the implant surface [120]. The accumulation of acidic substances is also evident, e.g., in renal system, wherein the epithelial tissue is adapted to withstand constant exposure to aggressive body-fluids. [119] Another source of chemical instability and a major concern with many implant materials is the composition of extracellular fluid, which contains high concentrations of Cl^- -ions that are acknowledged to have deleterious implications for the performance of many materials. The presence of these ions is particularly harmful with implant materials susceptible to corrosion because of the presence of considerable concentration of dissolved oxygen. The concentrations of ions in extracellular fluid are shown below in Table 5.1. In addition to electrolytes extracellular fluid is rich in metabolic substances such as active peptides, phospholipids, cholesterol and glucose [121].

Table 5.1. *Ion concentrations in human plasma and extracellular fluid, and concentrations of ions in simulated body fluid [122].*

	Ion concentrations / mM							
	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	HCO_3^-	HPO_4^{2-}	SO_4^{2-}
Human plasma	142,0	5,0	2,5	1,5	103,0	27,0	1,0	0,5
Simulated body fluid	142,0	5,0	2,5	1,5	147,8	4,2	1,0	0,5

5.1.1. Structure of bone and other connective tissues

In the human body the extracellular space is not solely consisted of extracellular fluid with dissolved molecules but also extracellular matrix which is composed of solid phase structures of various spatial forms. The specialised cells evolved from mesenchymal stem cells such as osteoblasts and chondrocytes secrete matrix constituents e.g., collagen, elastin, proteoglycan and glycoprotein molecules into extracellular space where the deposit is simultaneously decomposed e.g., by osteoclasts or macrophages, accounting for continuous remodelling of extracellular matrix. However, there are number of other factors that affect the equilibrium, i.e., the rate of anabolic and metabolic reactions, such as acid-base balance, calcium concentration, and hormonal regulation. [123] [124]

Regarding cold sprayed coatings, generation of extracellular matrix structures such as tendons, ligaments, bone, cartilage, enamel and dentine are of particular interest due to the applicability and potential of this process to manufacture hard-tissue implants. Hence, a certain degree of understanding of the structure and organization of connective tissues is necessary. Figure 5.3. is a hierarchical representation of bone structure and organisation of the collagen fibres, which highly determines its mechanical properties.

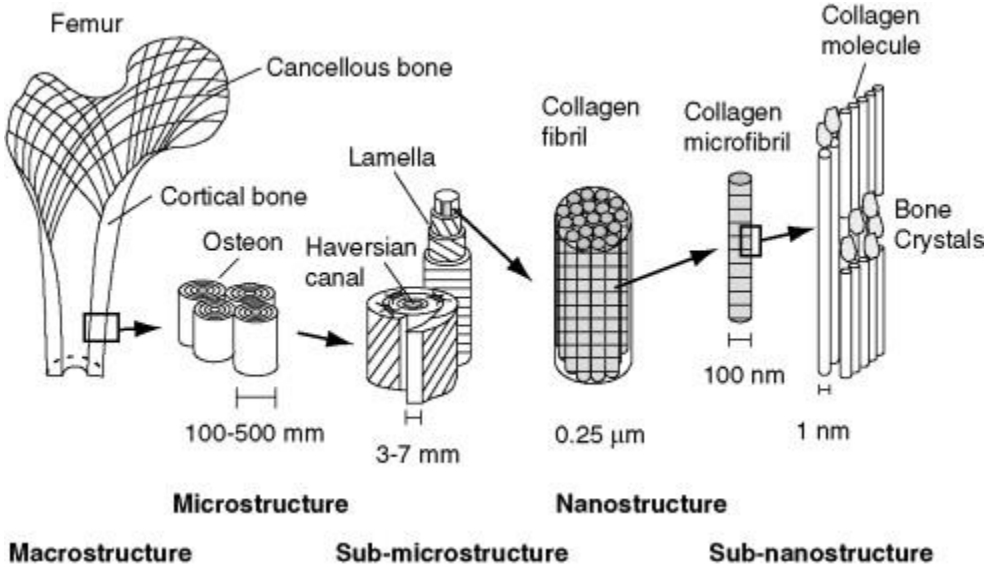


Figure 5.3. *Structural hierarchy of bone [117].*

Collagen proteins are the main constituents of bone accompanied by non-collagenous proteins such as osteonectin, osteopontine and osteocalcin. As opposite to other connective tissues bone structure is dominated by mineralised compounds of calcium, e.g., $Ca_{10}(PO_4)_6(OH)$, that interlock the proteins and provide excellent durability under

compressive stresses. The mechanical properties are dependent on the degree of protein frame alignment varying from longitudinally aligned cortical bone to randomly oriented cancellous bone. As a porous and metabolically active tissue, the bone is also vascularised. In contrast, the synthesis of other connective tissues such as tendons, ligaments, or articular cartilage is independent on mineralization and as a result, these tissues display higher degree of elasticity, ultimate tensile strength but modest durability under compression excluding cartilage residing in joint surfaces. [125] These biomechanical properties are profound for survival of the living cells as will be discussed in the succeeding section. Tensile properties of non-osseous connective tissues are shown in Table 5.2. wherein the tensile strength and strain are clearly influenced by the organisation and composition of the fibres.

Table 5.2. *Tensile properties of connective tissues [126].*

Material	Ultimate tensile strength (MPa)	Ultimate tensile strain (%)	Collagen (%/dry weight)	Elastin (%/dry weight)
Tendon	50 - 100	10 - 15	75 - 85	< 3
Ligament	50 - 100	10 - 15	70 - 80	10 - 15
Aorta	0,3 - 0,8	50 - 100	25 - 35	40 - 50
Skin	1- 20	30 - 70	60 - 80	5 - 10
Articular Cartilage	9 - 40	60 - 120	40 - 70	-

5.1.2. Understanding tissue response

The cells of the vertebrates are covered by a double layer of phospholipids, which are incorporated with hydrophobic ends of the molecules resulting in hydrophobicity of the cell membrane. The hydrophobicity of the membrane diminishes the permeability for hydrophilic substances but allows the liposoluble molecules to easily enter the cell. Hence, as the majority of the signalling pathways employ active hydrophilic molecules, the vital communication mechanisms of a cell with its surrounding include secretion and uptake of messenger molecules by endocytosis and exocytosis, binding of incoming signalling molecules with specific receptors, and incorporation of transmembrane proteins with extra cellular matrix. [118] The latest one: transmembrane protein activation plays a central role in integration of implant-tissue interface, as seen in Figure 5.4., because this signalling pathway is critical for normal cell functions and is only deployed when sufficient level of cell attachment onto surface exists. For this reason, the understanding on interactions between the transmembrane proteins and the surface is crucial from the point of view of biomaterial engineering.

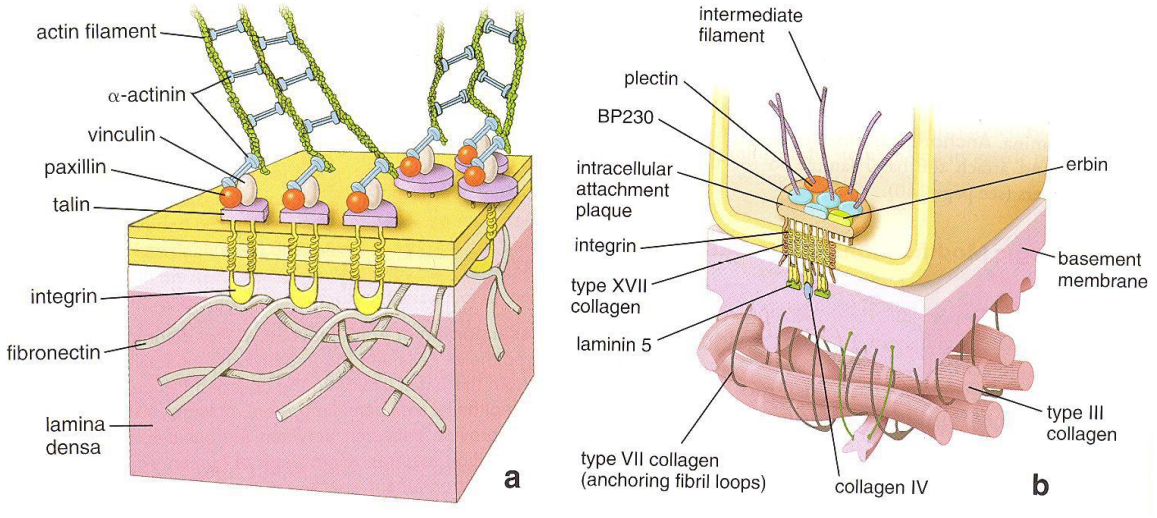


Figure 5.4. *Molecular attachment of cytoskeleton to ECM through a) focal adhesion and b) hemidesmosome [118].*

The group of transmembrane proteins responsible for linkage between cell and extracellular matrix include, e.g., the family of integrins, lectins, and proteoglycans. The relative amounts of these proteins are specific for each type of cell, i.e., vary because of different gene expression, which accounts for differences in affinities between different cell types and surfaces. As a consequence, the overall biocompatibility is a function of the structure of cell membrane, and for that reason, there is no universally suitable surface. In the biological environment extracellular end of the integrin protein forms connecting bonding with a large network of extracellular matrix consisting of fibrous proteins such as collagen, elastin, fibrillin, fibronectin, and laminin, which act as biomechanical stabilizers providing resistance for stretch, bend, torsion and compression. Following the activation of integrin the intracellular end of these proteins is bound with cytoskeleton, which consists of intracellular proteins, such as, actin and myosin, and physically supports the cell structure and determines the characteristic biomechanical properties for each type of cell. [118] [127] Precisely, the binding of integrins into any surface is not only a matter of cell attachment but has much more fundamental implications for the cell functions as illustrated in Figure 5.5. Consequently, the binding of integrins with ECM regulates the survival, migration, proliferation, and differentiation of the host cell and accounts for the cell death in case of inadequate attachment. Therefore, much efforts has been made to unravel the exact underlying mechanisms of the binding of integrins and attachment of cells to ECM, but are not yet thoroughly crystallised. From the perspective of biomaterials engineering the aim is to develop biomimetic materials, which show similar biochemical, biophysical, and biomechanical properties to natural tissue. [128]

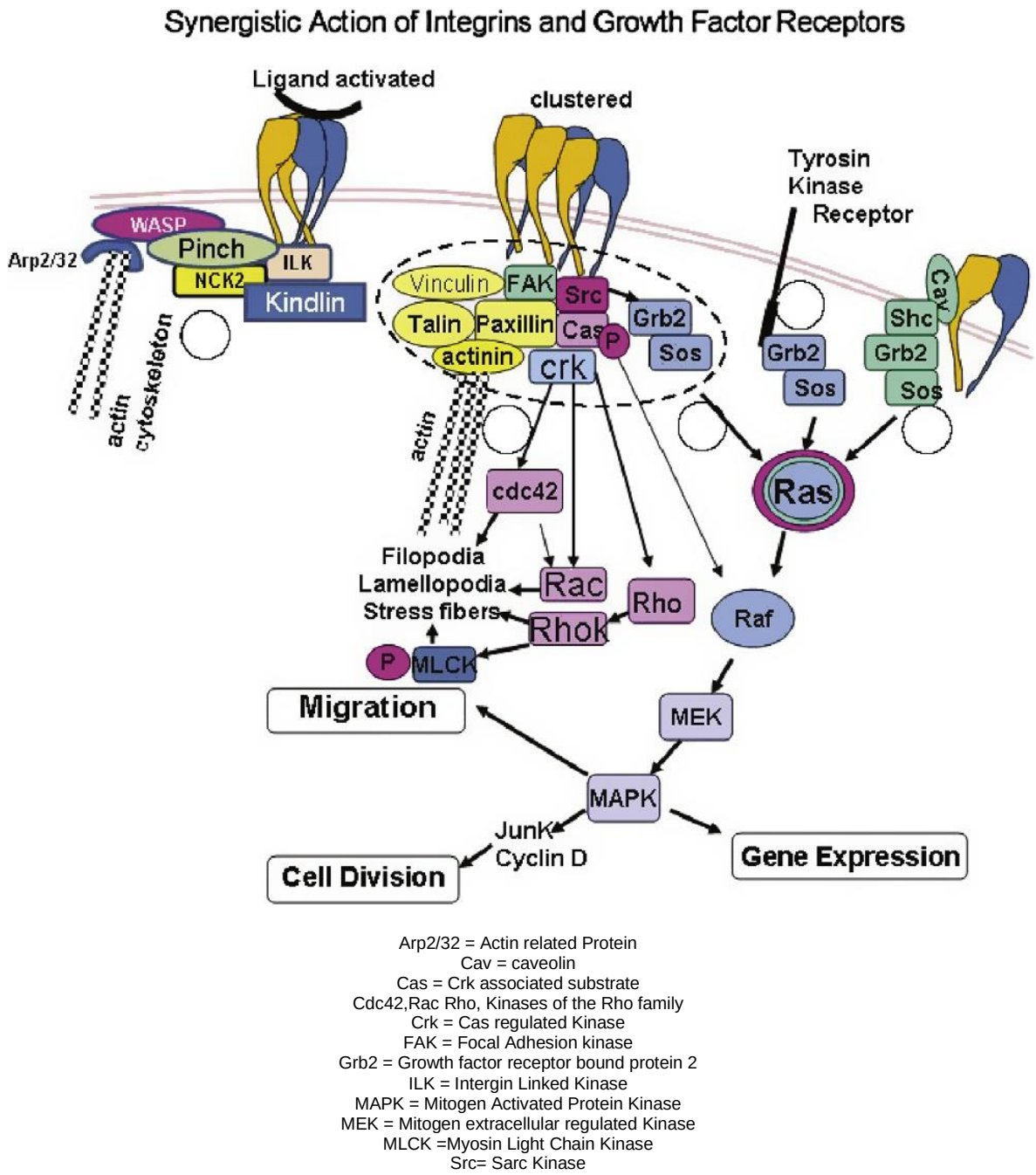


Figure 5.5. *Integrin-activation dependent signalling pathways. Transmembrane integrin is a key player in mediation and regulation of gene expression and cytoskeleton organisation through ligand activation, clustering, and tyrosine kinase receptor [128].*

The attachment of integrated membrane proteins to adhesive extra cellular matrix proteins depends on biochemical, biophysical and biomechanical factors. Firstly, the peptide sequences between the surface proteins and ECM proteins are highly selective and are only able to bind epitopes with complementary chemical structures. With the biomaterials the

most well-established epitope used for anchoring the cell proteins is a tripeptide known as RGD, which is capable of recognising certain integrin sub-groups. [129] Also, controlling the chemical properties such as surface charge and hydrophobicity enables the modification of protein binding on the surface of biomaterials. The contribution of biophysical surface topography on the integral membrane protein –mediated attachment has been suggested for some materials, and the cellular response has been concluded to correlate with pattern and spacing of adhesive ligands. Most essentially, the topography with an abundant amount of fine binding sites with regular structure did facilitate the integrin attachment less than similar nanostructure with extended gaps between the assumed sites of binding. [81] Therefore, current theory that is underpinned by the former finding, proposes that the density of topographical binding sites should equal to the density of adhesive proteins in order to maximise the number of bondings between the targeted protein couple to near-theoretical values. The most prominent biomechanical properties are the elasticity and resilience because of the predominant effect of these properties on cell migration, proliferation and differentiation. As mentioned before, the regulation of these cell functions is mediated by integrin activation, and due to the fact that ECM is connected to intracellular microfilament network via integrins, movements of ECM induce and maintain the cell functions. This also has an effect on cytoskeletal organisation and the shape of a cell, which in turn might have relevance when, e.g., interpreting experimental results of biomechanical compatibility. Nevertheless, in order to obtain such biomimetic modulatory effect on cell functions the resilience and elasticity of the matrix comparable to biological tissue are vital. [128] However, the regeneration and growth of a cell is also stimulated by growth factors and hormones that act synergistically with integrin activation in biological environment [127]. In addition, as with topographical features, each type of cell is adapted to interact with a matrix with specific mechanical properties, and placing on a matrix with incompatible biomechanical behaviour disrupts the cell renewal and growth or might even lead to cell apoptosis in some cases, such as, chondrocytes placed on a hard substrate. [128]

5.1.3. Protein adsorption

In an extra cellular matrix there are proteins inherently present. However, with artificial biomedical implants the surface is usually free of such proteins at the initial state. Hence, the adsorption of the proteins of the extracellular fluid onto implant surface is vital since it constitutes the first step in the process of tissue regeneration. The accumulation of surface proteins occurs within a temporal frame of few minutes after the introduction of implant to body fluid and is accompanied by activation of reaction cascades resulting in cell attachment during the following hours. However, whether the protein adsorption is desired or unwanted depends on its implications in the particular application, e.g., with osseoinducing bone implants the promotion of cell attachment is advantageous whereas with cardiovascular implants any debris on the surface is unacceptable because of increased probability for blood

cell aggregation and subsequent stenosis. [130] Non-fouling surfaces are also pursued when designing medical devices such as surgical instruments, urinary catheters, vascular catheters, biosensors, haemodialysis membranes, gas-exchange membranes, cerebrospinal shunts, and intrauterine contraceptive devices. [49] [131] [130] With respect to protein rejecting implants, protein adsorption kinetics and its thermodynamic control are important aspects to consider in order to preventing the accumulation of such proteins [132]. These factors in turn are associated with adsorption mechanisms and surface structure.

The proteins are adsorbed in a solid surface mainly by two mechanisms: electrostatic and hydrophobic interactions between the surface and the charged functional groups, which accounts for pronounced role of surface properties such as surface charge and wettability in protein adsorption. [133] Depending on these properties the protein molecule experience repulsive or attractive forces at the vicinity of the surface. Usually, the repulsion dominates preventing pathological entanglement of body proteins but once the protein passes thermodynamical barrier it couples to a surface and reaches an energetically stable distance, which is illustrated in the Figure 5.6. where Gibbs' free energy is shown as a function of separation distance between the surface and the protein. [130]

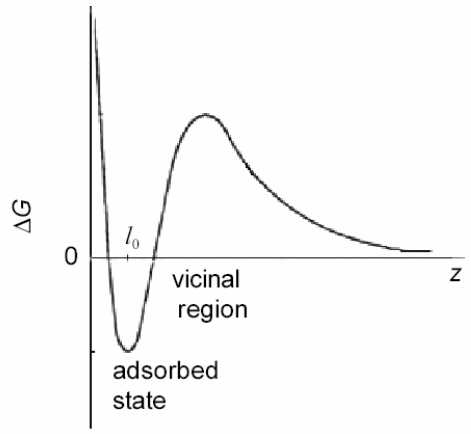


Figure 5.6. *The influence of separation distance on interfacial potential, equilibrium distance l_0 being ~0,15 nm [130].*

The configuration of a single amino acid varies according to prevailing pH. Therefore, high number of different protein configurations exists and, as a consequence, the binding affinity of each protein with an altered charge, is unique. This is a fact that has to be considered particularly, when surface charge of an implant is under investigation. The changes in the charge of an amino acid histidine is shown as a curve in Figure 5.7.

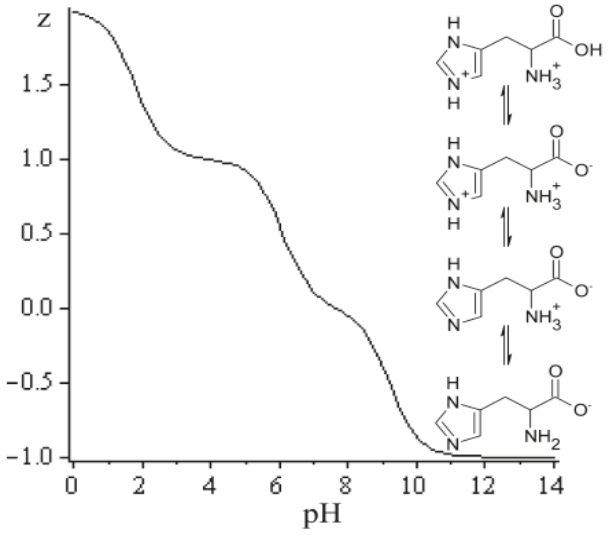


Figure 5.7. *The charge alters along an increasing pH as characteristic to amino acids such as histidine [134].*

The influence of chemical surface structure on adsorption of prevalent extracellular proteins such as fibrinogen, fibronectin, albumin, IgG., has been demonstrated in a number of studies. Especially surfaces with functional groups such as carboxyl (-COOH), hydroxyl (-OH), amine ($-NH_2$), and methyl ($-CH_3$) have been constructed in experiments in order to modify the protein affinities resulting from different levels of hydrophilicity and surface charge displayed by these functional groups. Altogether, the proteins tend to respond differently to altered relative quantities of these functional groups at the surface and as a consequence, also the cellular responses vary according to the cell type. [135] Consistently, the surfaces with metal oxide structure exhibit distinct protein adsorption characteristics, which will be elaborated in chapter 4.3.4. as wettability and surface charge are discussed. [133]

In the past the protein adsorption in the biomedical implant surfaces highly relied on “trial and error”-experiments. This fact mainly attributes to high unpredictability of the cellular response, which fundamentally arise from poorly understood and extremely complex protein adsorption phenomenon. Particularly, the conformational changes in protein structure that take place during adsorption, as illustrated in Figure 5.8, result in exposure of epitopes, which are hidden prior to transformation. Such epitopes are able to act as binding sites for proteins previously incapable of coupling to [135]. Therefore, the simulation of the reaction chains of accumulating proteins is challenging and one of the underlying causes for why systematic approach is often ignored in assessing biocompatibility.

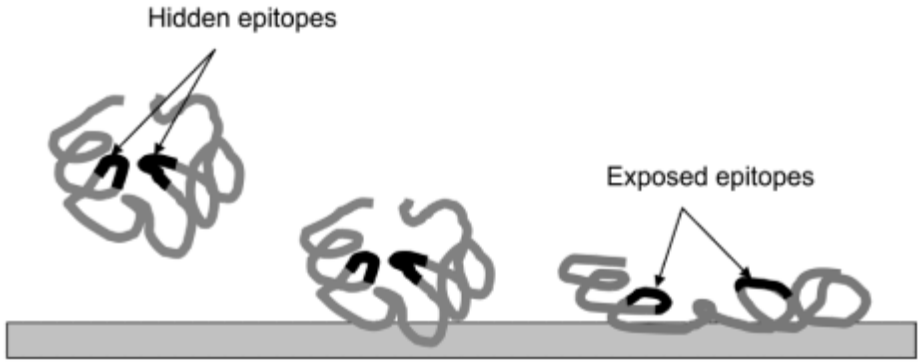


Figure 5.8. *During unfolding of the helical structure on the implant surface, fresh epitopes are introduced and might act as antigenic binding sites [135].*

Despite a low level of understanding of protein interrelationships, a few established mechanisms needs to be expressed. As for kinetics, one of the dominating theories suggest that the progression of the protein adsorption occurs in stepwise manner in a process known as Vroman effect wherein the surface is preferably occupied by light proteins initially, that are gradually displaced by proteins with higher molecular weight or by proteins with stronger bonding [136] [137]. However, the process is not exclusively a matter of biomaterial surface and protein interaction but actually, is governed by the water molecules, which are involved with protein adsorption in excessive quantities. At present the perception of competitive nature of protein adsorption phenomenon between water and protein molecules has been recognised. It is also worth mentioning that there are still many controversial issues related to protein adsorption that lack definitive answers such as reversibility and irreversibility of protein adsorption, multilayered agglomeration, and specific mechanisms of Vroman effect. [138] Altogether, as the adsorbed proteins on an implant material form an artificial extra cellular matrix for the overlying cells to attach and proliferate, a careful consideration of surrounding tissue is needed when designing implant surfaces.

5.1.4. Immune responses

According to a classical view for a few decades ago a concept of biocompatibility was solely based on the ability of material to withstand in vivo environment without being degraded. Hence, the biocompatibility was perceived as an inability of the implanted material to induce hazardous responses and the main goal was to prevent any immediate or long-term adverse effects. For that reason, the focus was on developing inert materials with non-toxic, nonimmunogenic, non-thrombogenic, non-carcinogenic, non-allergenic properties, which mostly are yet valid requirements for medical devices in these days. Nonetheless, the idea of biological safety of an implant material is inadequate pre-requisite at present as an increasing amount of energy is directed to improve the biocompatibility in order to evoke appropriate response. [131] This chapter further outlines the basic immunologic responses, which are

central to understand when highly sophisticated biomaterials are addressed. Instead, the precise mechanisms of some reactions, e.g., carcinogenicity, are omitted here as they are beyond the scope of this review.

In a case of favourable tissue response, the displacement of an original tissue with a substitute is immediately followed by blood-material interactions resulting in coagulated provisional matrix formation and subsequent acute inflammation characterised by arrival of mast cells and histamine-assisted infiltration of leukocytes, particularly monocytes. The type of inflammation undergoes a shift from acute to chronic as the amount of clustered monocytes and lymphocytes increase, which usually leads to fibrous capsule formation and wound healing beyond three weeks. The infection is indicated by the persistence of inflammatory response for more than three-week period. However, incorporation of a poorly biocompatible implant into in vivo environment elicits a non-specific foreign body reaction, which is a protective response aiming at removal of the material by phagocytosis. In this action particles are being attached, surrounded and degraded by macrophages, but their capability to phagocytose particles is restricted to around 5 μm particle size while larger particles with diameter over 10 μm induce foreign body reaction. The initiation of such reaction is triggered by specific proinflammatory agents such as interleukins released from mast cells and lymphocytes. [120] In this process, called chemotaxis, substances facilitate monocyte migration through endothelial barrier towards the surface and promote the adhesion onto freshly adsorbed layer of proteins [139]. Among the proteins absorbed onto surface fibrinogen, complement, and antibodies show highest involvement in foreign body reactions playing a key role in recruitment of the monocytes [140].

As mentioned earlier, the conformational changes in protein structure during adsorption often have a radical impact on binding affinity of other proteins including a number of epitopes involved in the immune system. An acute response is triggered by freshly exposed antigenic epitopes misinterpreted and recognised by the cells, e.g., monocytes, dedicated to activate the defence system. A representative example of that is fibrinogen, which undergoes conformational changes during adsorption onto surface and two different proinflammatory domains, epitopes P1 and P2, are exposed that are normally hidden in its solute form. These epitopes are attractive binding sites for macrophages and thus, underpins the central role of fibrinogen adsorption for initiation of foreign body reactions. [139] The attachment and activation of the monocytes is followed by differentiation to macrophages that further fuse together to form foreign body giant cells characteristic of foreign body reaction as Figure 5.9. suggests.

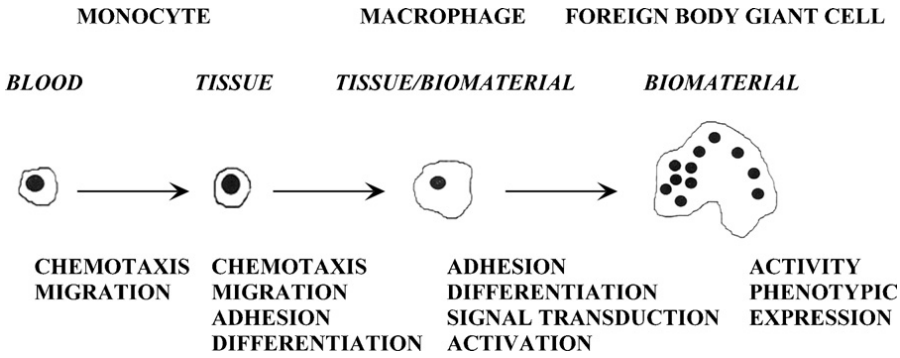


Figure 5.9. *Development of monocyte to foreign body giant cell on biomaterial surface [120].*

From the perspective of biomaterials engineering, the possible consequences of the foreign body reaction to material performance must be considered since there is a wide range of studies proving the link between deleterious degradation of material properties and foreign body reaction. The degradative enzymes, reactive oxygen intermediates (ROIs), and acid produced by macrophages and foreign body giant cells has been identified the reason for degradation since they concentrate the zone between the cell membrane and biomaterial surface resulting in aggressive microenvironment. However, macrophages have a contribution to fibrosis in normal wound healing increasing the number of fibroblasts at the vicinity of macrophages. [120] The mediators produced by biomaterial adherent macrophages are mainly responsible for accelerated proliferation and upregulated number of fibroblasts that become attracted and activated leading to intensified fibrogenesis and formation of extra-cellular matrix around the foreign body. [141] Figure 5.10. illustrates a typical foreign body granuloma surrounded by ECM. As the synthetisation of extra-cellular fibrous connective tissue proceeds the macrophages are gradually replaced by external ECM resulting in encapsulation and partial isolation of biomaterial. Unfortunately the contraction of the fibrous capsule is frequently associated with painful complications as well as biomaterial dysfunctions. [120] [142] Altogether, these practical implications arise from the lack of attachment between the human cells and implant surface which might be caused by several reasons, such as, deficiency in protein adsorption or insufficient integrin binding. These factors might further originate in the shortages of biochemical, biophysical, and biomechanical properties of the implant surface.

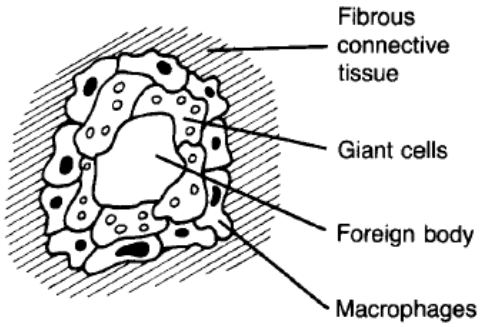


Figure 5.10. *Foreign body granuloma, which is frequently observed at the vicinity of biomaterial surface [142].*

In addition, there are a few other, either immediate or long-term, adverse effects the biomaterials might have on in vivo environment such as pyrogenicity, carcinogenicity, and allergenicy i.e. acute or delayed hypersensitivity which all are expressed by various macroscopic symptoms. The apparent symptoms might also result from cytotoxicity which is defined as the probability of a substance to cause cell death.

5.1.5. Bacterial infections

An implantation of any medical device holds a serious risk of bacterial infection. Collective data from U.S. hospitals reported in 2007 revealed two thirds of nosocomial infections were related to implanted medical devices. Thus, prevention of infections is one of the major problems unresolved. The colonisation of bacteria onto surface resembles the recruitment of mammalian cells as it is also assisted by the adsorption of the same proteins. However, in comparing with mammalian cells a special feature of bacterial cells is the formation of biofilm consisting of insoluble gelatinous exopolymers secreted by bacterium that is difficult to remove. Prolonged bacterial infection in turn might maintain chronic inflammation leading to systemic complications. [143] [144] Therefore, in terms of biomaterials, antibacterial coatings loaded with some selectively toxic substance are now being pursued.

5.2. Mechanical properties of implants materials

The characteristics of the thermal spray processes, especially the fact that relatively thick coating is formed, unavoidably accounts for the changes to mechanical properties of the substrate material whenever these processes are applied. Whether there is an improvement or reduction detected in mechanical properties of an implant during the coating process is an important factor defining the attractiveness of the coating. Particularly with coated hard-

tissue implants such as fixation plates and screws which are used in load-bearing applications, the mechanical properties gain extra attention because the entire existence and purpose of tissues such as bone, cartilage, ligaments, dentine, enamel etc., is based on their mechanical functionality. Meanwhile, the mechanical failures are a considerable threat for well-being of an individual either directly in loss of the intended function or indirectly causing an inflammation or another undesired reaction. In order to assess the mechanical viability or performance of a hard-tissue implant, Table 5.3. demonstrates the typical values of mechanical properties of most widely used or most promising biomaterials for load-bearing applications.

Table 5.3. *Properties of the essential biomaterials. Compiled from references [44] [59] [81] [85] [145] [146] [147] [148] [149].*

Material	Young's modulus	Tensile strength	Fracture toughness	Compressive strength	Hardness	Density
	GPa	Mpa	Mpa $m^{1/2}$	MPa	HV	g/cm^3
Bone	12 - 21	60 - 130	3 - 6	130 - 180		1,3 - 2,1
AISI 316L	190	930 - 1350	50 - 200	170 - 310	150 - 190	8
Ti6Al4V	110	900 - 1200	55 - 115	758 - 1117	350	4,4
Co28Cr6Mo	210	650 - 1600		450 - 1000	270 - 350	8,3
Ti30Nb/Ti30Ta	60 - 80	700 - 750				
Mg	41 - 45	230	15 - 40	65 - 100		1,7 - 2,0
α-Al_2O_3	400	200-300	4 - 5	4250	2400	3,96
ZrO_2 (Y-TZP)	150		6 - 15	2000	1200	5,6
$Ca_{10}(PO_4)_6(OH)_2$	70 - 120	35 - 45	1	350 - 520	500 - 800	3,1
Bioglass 45S5	30 - 35				500 - 600	2,7
UHMWPE	1 - 3	41				0,95
PMMA	3	80				
PEEK	3	92				1,3

In an average case mechanical failure takes place as a result of fatigue after a number of stress cycles encouraged by corrosion and wear. The occurrence of such failures is influenced by mechanical properties of the implant, site of implant, age of the patient, lesion type, etc., and is often followed by a complicated revision surgery. The hard-tissue failures are most often reported posterior to total hip or knee replacement operations but are apparent with all the hard-tissue implants such as spinal components [150]. Altogether, the complex nature of biomechanics as well as macroscopic mechanical aspects of hard-tissue implants are

discussed here with an emphasis on cold sprayed coatings that hold a promise to remedy implants inflicted by fatigue, corrosion and wear.

5.2.1. Tensile and compressive strength

Tensile strength is a valid indicator of an overall material strength. As seen above in the Table 5.3. the tensile strength values of the metallic endoprosthetic materials are far superior to those of natural bone whereas of polymers and ceramics show greater correspondence to tensile strength values of natural bone. Thus, the popularity of metallic implants in load-bearing applications results from the fact that the failure mechanisms are much more favourable than with brittle ceramics, which typically undergo a sudden crack growth and final fracture as the durability limit is exceeded. As for polymers, a failure occurs in more controllable manner and in conjunction with mechanical values close to natural bone has become one of the motives for ongoing research. Altogether, an adequate tensile or compressive strength is relatively easy to reach and hence, the challenge is to sustain the mechanical strength under corrosive, wearing, aqueous environment when, at the same time, the inserted material is constantly subjected to cyclic loading.

Presumably cold spraying of hard particles on polymeric substrate might result in reduced tensile strength due to plenty of crack initiation sites generated by embedded particles. Also, the ratio of applied/critical –stress of the polymers used in load-bearing medical devices is often considerably higher than with metal or ceramic substrates [151]. Thus, lower-than-expected tensile strength might follow when soft polymeric substrates are subjected to cold spraying. However, the strength of cold sprayed coatings on polymer substrates is devoid of scientific demonstration.

Typically there is a reduction in tensile strength as a consequence of depositing powder processed coatings due to abundance of potential crack initiation sites provided by porous coating microstructure. This was manifested in the study of Vo et al. [29] by higher strength of the He-processed dense coatings in contrast to N_2-processed porous coatings expressing lower strength. However, in terms of strength, porosity level has been displayed a moderate link to tensile strength although more pronounced role of inter-particle bonding has been pointed out. [29] Therefore, the effect of annealing on diffusion-driven bonding has been a subject of studies on the field of mechanical behaviour of cold sprayed coatings. Annealing was earlier on tested with cold-sprayed Cu coatings by Gärtner et al. [152] with an enhancement in tensile strength recorded. The improvements in strength have recently been demonstrated with cold-sprayed coatings deposited onto biomedical alloys after annealing treatment compared to cold sprayed coatings tested in as-sprayed condition. Stainless steel 316L was coated with a mixture of steel and Co-Cr particles by Al-Mangour et al. [40] who reported a significant enhancement in stress-at-failure proportional to annealing temperature. These findings are consistent with the results published by Vo et al. [29] for Ti-6Al-4V alloy

cold sprayed onto Ti-6Al-4V substrate. However, the net tensile strength did not reach the strength values measured for as-received Ti-6Al-4V alloy as presented in Figure 5.11. but as a whole, are far superior to strength values suggested in Table 5.3. for natural bone and are easily outweighed by properties such as fatigue resistance. Tensile test results for as-received Ti-6Al-4V alloy (curve 1), low-porosity Ti-6Al-4V alloy cold spray deposited onto Ti-6Al-4V substrate (curves 2&3), moderate-porosity Ti-6Al-4V alloy cold spray deposited onto Ti-6Al-4V substrate (curves 4&5) are show as a graph in Figure 5.11.

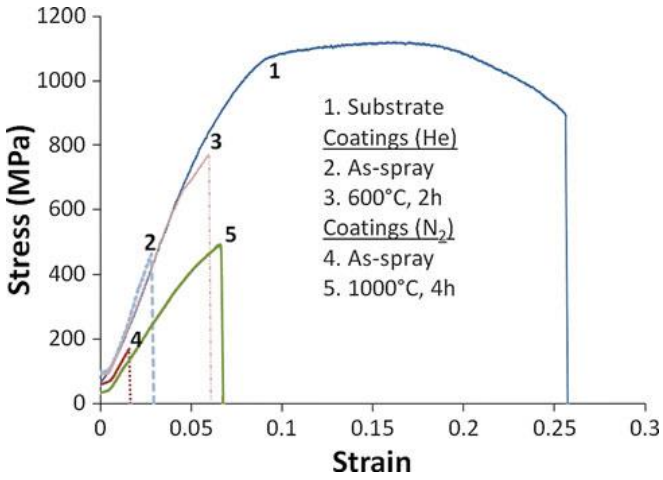


Figure 5.11. *Stress-strain curves of Ti-6Al-4V in* ***1.*** *As-received condition,* ***2.*** *Cold-sprayed Ti-6Al-4V, processed with He-gas,* ***3.*** *Cold-sprayed Ti-6Al-4V, processed with He-gas, annealed* ***4.*** *Cold-sprayed Ti-6Al-4V, processed with N_2-gas,* ***5.*** *Cold-sprayed Ti-6Al-4V, processed with N_2-gas, annealed [29].*

As directionally opposite to tensile stress the durability of a material under compressive stress is expressed by compressive strength which limits hard-tissue applications. However, it is typically detrimental only when combined with wearing under cyclic loading and hence, is to be dealt with in the following chapter.

5.2.2. Fatigue endurance

Fatigue, being particularly relevant to metals, is a phenomenon that takes place as a result of multiple stress cycles and might cause a premature implant failure and mechanical collapse due to loss of strength subsequent to gradual crack propagation. In context of fatigue-induced crack initiation and growth, surface finish and coating microstructure play a key role. Therefore, the impact of surface treatments and coatings on factors behind the fatigue resistance has been a subject of many investigations. [153] Under *in vivo* environment the fatigue often acts concurrently with corrosion and wear on mechanical properties of the inserted biomaterial and, for this reason, corrosion-encouraged and wear-induced fatigue are essential concepts to consider.

From the perspective of coatings engineering the fatigue is a major concern since the degree of susceptibility to failure induced by fatigue is highly dictated by defects such as microstructure heterogeneities or micro-cracks. In terms of coating properties porosity, oxide content, inter-particle cohesion, interface adhesion and surface roughness are central. However, the fracture mechanism depends on the material properties and can be classified according to observations at the bulk surface such as plastic deformation, oriented cracks, or microcracking, as seen in the Figure 5.12. These types are found in the surface of bulk biomaterial, but also form the basis on understanding the fatigue behaviour of coatings. [154]

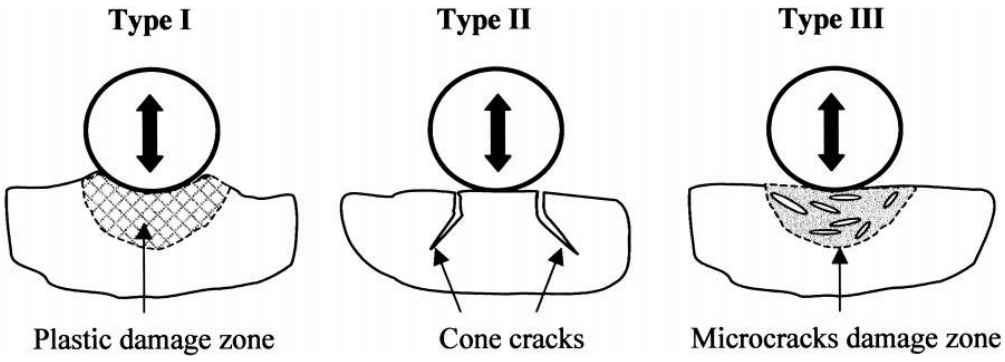


Figure 5.12. *Schematic illustration on the appearance of failure types characteristic under a spherical indenter for a material with high fracture toughness and high ductility (type I), high strength and low fracture toughness (type II), and moderate strength and toughness (type III) [154].*

The generation of deteriorating defects, such as microcracks or microstructure heterogeneities, is promoted by the presence of grooves at the surface as a result of wearing, or increased surface roughness due to corrosion. Fretting is the most prevalent phenomenon that falls into an umbrella of wearing and often causes dramatic reduction in fatigue strength. It is involved in microvibration processes applied with compressive loading wherein microcracking which is associated with high exerted shear stress takes place. The presence of excessive amount of crack initiation sites gives raise to likelihood of crack propagation and abrupt failure as the impact of stress cycles accumulate. [155] Activation of corrosion fatigue mechanism is based on cyclic mechanical loading which plastically deforms the subsurface slip planes and metal surface leading to exposure of the fragmented oxide layer to aggressive environment as shown in Figure 5.13. The rate of repassivation is inadequate to restore the uniform oxide layer, which accounts for the fact that the unoxidised surface domains are being attacked by corrosion and is often responsible for impaired resistance for fatigue because of the nucleation sites provided by corroded uneven surface. Moreover, as many of the actual implant surfaces are continually exposed to a combination of corrosion and fatigue these phenomena are often stated as corrosion fatigue. [153] [154]

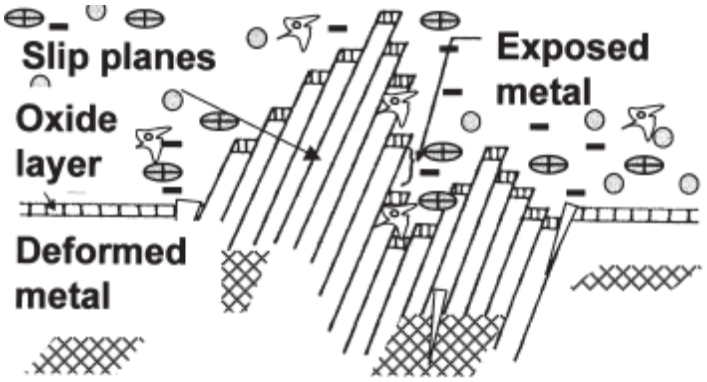


Figure 5.13. *Plastically deformed metal surface, which is prone to corrosion in the physiological environment due to fragmented oxide layer [154].*

Predominance of localized thermal fatigue has been deduced as the main failure mechanism for biopolymers due to low thermal conductivity and ductile behaviour displayed by the materials of this group. Although the polymers are rarely used as load bearing hard-tissue substitutes some of them such as PEEK and polysulphone (PS) have shown their potential in endoprosthetic applications. However, some computational models have predicted fatigue strength as low as 12 MPa for bulk UHMWPE, which has become the dominant design in acetabular cups of hip prostheses wherein the actual loading might exceed 30 MPa. In contrast for bulk PS and PEEK the values were 45 MPa and 75 MPa respectively. [154]

The modes of failure frequently observed at the thermally sprayed cermets and ceramics include abrasive fatigue, delamination, bulk failure and spalling. Firstly, abrasive fatigue was reported as the primary failure mechanism of thermally sprayed cermet or ceramic coatings in rolling elements by Ahmed et al. [156] with indications of asperity contact induced by microslipping and sliding at the contact region, which is associated with surface micropitting and wear. Also, high local stress concentrations were observed with respect to asperity contacts which accounts for the susceptibility of hard coatings to microcracking and fatigue. In the *in vivo* environment however, lubrication reduces the abrasion rate and, for example, fretting fatigue of Ti alloys in simulated body fluid is dampened regardless of corrosive environment by the lubricative fluid [47]. Secondly, delamination of a coating originates from microcracks residing at the coating-substrate interface which expand and coalesce over time during cyclic loading. In investigations conducted by Laonapakul et al. [53] this mechanism has been linked to plasma sprayed HA coatings with which fatigue cycles exerted prior to failure showed strong dependence on incorporation of immersion in simulated body fluid. The findings are found to be consistent with results published earlier on by Gledhill et al. [84] who argued for reduced fatigue life in simulated body fluid (Ringer's solution) with plasma sprayed HA. Accelerated dissolution of soluble HA phases was stated as the main reason for changes in coating structure and increased tendency to fail. Moreover, HA

deposited with detonation gun exhibited preferable fatigue resistance in comparing to plasma sprayed coatings with lower extent of delamination due to better adherence onto substrate. In addition to these studies conducted under *in vitro* environment, delamination has been recognised as a considerable clinical problem of implants coated with plasma sprayed Ti and HA [157] [158] [159] [160]. The third scenario, bulk failure mechanism, is characteristic for combination of hard coating on a soft substrate leading to plastic deformation of the substrate while the coating remains in its elastic range. Eventually, adhesion between coating and substrate is lost which results in rupture of the top layer. Spalling is a type of fatigue failure, which occurs under high load exerted on a small area and is expressed by scaling of the coating layer usually along wear tracks. [156] In addition, a dependency between spalling and elastic modulus has been deduced by Ibrahim et al. [78] with HVOF-sprayed nanostructured TiO_2 coatings on steel substrate.

As mentioned earlier, the compressive residual stress of the cold sprayed coatings along with nonporous, low-oxide structure have been considered as ideal properties to prevent fatigue. There have recently been some studies looking at the fatigue behaviour of cold sprayed coatings with ambivalent outcomes. Regardless of positive premises, Price et al. [50] examined cold sprayed Ti coatings on Ti6Al4V and described a "detrimental effect on fatigue endurance limit". The reduction was believed to result from variations in surface topography and substrate tensile residual stresses emerging from the spraying process as well as insufficient compressive stresses within the coating structure. Consistent reduction in fatigue strength with the same materials was confirmed by Cizek et al. [27] in cyclic-bend tests with fracture initiation from longitudinal coating cracks. Similar trend was demonstrated by Al-Mangour et al. [38] with cold sprayed, substrate-free stainless steel 316L coating, wherein annealing post-treatment, conducted in order to promote diffusion bonding, failed to restore the fatigue resistance equivalent to bulk material. On the contrary, substantial improvement of fatigue endurance of Al alloy 2024-T3 (Al4Cu1Mg) was reported by Sansoucy et al. [161] owing to contained compressive residual stresses of the cold sprayed Al-Co-Ce coating, which prevent nucleation and propagation of cracks. Along with compression within the coating structure, the fundamental role of excellent adhesion onto substrate was identified as the coating-substrate interface remained intact with no delamination detected. A prolonging effect on fatigue life was also observed by Ibrahim et al. [78] with nano-structured TiO_2 coatings sprayed with HVOF onto AISI 1018 substrate. The improvement of fatigue resistance in comparison with traditional plasma sprayed coatings was inferred to result from nano-structuring of the powder as well as compressive residual stresses induced by HVOF process.

Optimistically speaking some enhancement in the fatigue endurance limit might be achievable by means of cold-sprayed coatings, but optimal spraying parameters are required since the experiments done with traditional biomedical materials: stainless steel and Ti, have

both shown unexpectedly low resistance under cyclic stresses. Considering the fact that with plasma sprayed coatings, which are widely utilised in biomedical field, delamination of the coating on the one hand, is one of the present challenges and on the other hand, a bond coat e.g. HA/Ti bond coat between Ti substrate and HA top coating, is needed according to Laonapakul et al. [54] to overcome poor fatigue resistance, puts the cold spraying into another perspective. In addition, the impact of annealing on bonding strength emphasises the importance of inter-particle as well as coating-substrate interface adhesion on fatigue endurance.

5.2.3. Adhesion between coating and substrate

Inter-particle cohesion and coating-substrate interfacial adhesion, are material properties specific to sprayed coatings. Weak bonding, as discussed in the previous chapter, often precedes the detachment of coating and substrate interface, which might have serious consequencies as revealed by several clinical studies [157] [158] [159] [160]. However, it is only possible for such deterioration of conformity to take place in the presence of mechanical loading assisted by corrosive or wearing circumstance and is affected by spraying parameters as well as particle size and shape [51]. Especially the mechanical instability of the coating-substrate interface has been reported, as evidenced by studies indicated above, in context of plasma sprayed HA coatings. A major limitation with respect to high temperature fabrication process of these coatings is the formation of amorphous phases prone to dissolving into physiological fluids or formation of chemically altered structures such as tricalcium phosphates, tetracalcium phosphates or calciumoxide. [162] [79] Therefore, a substantial decline in adhesion of the HA coating might follow, and is typically observed in dramatic extent within 24 hours [77] or on the course of few weeks [56] in an immersion test accomplished in vitro.

Because of the co-existence of adverse phase transformations and limited mechanical properties, more attention has been paid on spraying composite coatings containing HA with biocompatible reinforcing component, such as, Ti, TiO_2, YSZ etc., with techniques that make use of higher impact velocity. In using spraying techniques with lower thermal input and higher velocities, limited phase transformations occur with highly compacted coating that displays adequate adhesion and cohesion. HA-YSZ composite coating was prepared by plasma spraying by Yugeswaran et al. [57] with remarkably improved adhesion strength owing to decrease in the solidification rate of HA particles, which inhibits the formation of amorphous layers. Adding 30 wt% of YSZ doubled the adhesion strength of HA coating. Likewise, good adhesion strength was displayed by coatings successfully produced by plasma spraying with Ar gas [55]. In the investigations conducted by Gaona et al. [79] and Melero et al. [77] consistency has been found regarding the degradation of adhesion experienced by HVOF-sprayed TiO_2 coating with increasing proportion of HA. Cross-

sectional structure of HVOF-sprayed TiO2 and TiO_2 + 20 wt-% HA acquired by Gaona et al. [79] is illustrated in Figure 5.14. Adhesion strength values as high as 68±14 MPa for TiO_2+20 wt% HA, and over 77 MPa for TiO_2+10 wt% HA were recorded by Gaona et al. [79] whereas Melero et al. [77] stated adhesion values from 52 MPa to 64 MPa for TiO_2+80 wt% HA. However, the effect of adhesion degradation as a result of selective dissolution of certain impure phases derived from HA is only detected at the aqueous environment and hence, these preliminary results are to be considered somewhat of a trivial. To support the statement, a dramatic drop in adhesion strength from 52-64 MPa to values well below 20 MPa was recorded within 1 day of immersion in simulated body fluid with TiO_2+80 wt% HA [77]. Equivalent adhesion strength values have been observed with TiO_2+60 wt% HA by Ferrer et al. [163] after exposure to simulated body fluid. Even lower adhesion strength values have been proposed with plasma sprayed HA coatings subsequent to immersion test [77]. Nevertheless, the threshold value specified by ISO 13779-2:2008 Implants for surgery–Hydroxyapatite–Part 2–Coatings of Hydroxyapatite -standard for adhesion strength of implantable devices with HA coating is 15 MPa at a minimum [104].

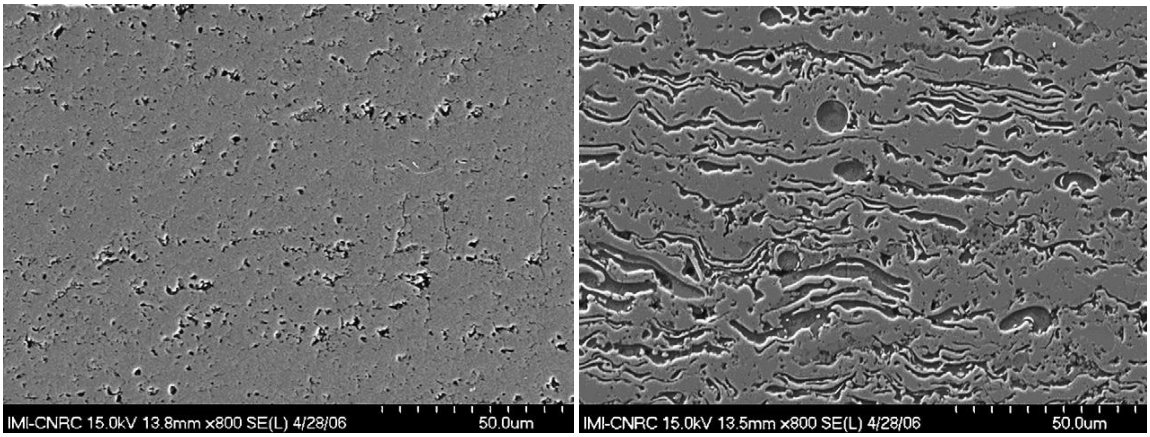


Figure 5.14. *Cross-sections of HVOF-sprayed nanostructured TiO_2 (left) and TiO_2 + 20wt-% HA (right). [79]*

Unconvincing adhesion strength of plasma sprayed coatings has also been a motivation to some studies on the applicability of cold spray process in producing HA coatings. Choudhuri et al. [162] fabricated Ti+30 wt%HA coatings by cold spraying with a selection of parameters, and demonstrated the correlation of cohesive strength and the emergency of porosity. Maximum averaged adhesion strength was 47 MPa in as-sprayed coating with dense structure. In addition, even with the lowest levels of porosity, the failure of coating was the preferred mode of failure indicating superior adhesive bonding in comparing to cohesive strength. In an attempt to overcome comparably low adhesion strength the annealing treatment has been recognised as a viable method [40] [29]. Qiu et al. [164], who created cold sprayed Ti/HA coatings with average porosity content of around 60 % and average pore

size of around 85µm, reported shear adhesion strength as high as 20 MPa. Porous Ti coating was also produced by Sun et al. [165] and reportedly, tensile adhesion strength of 42 MPa was confirmed. Similarly, tensile adhesion strength for a pure Ti on Al substrate was 81 MPa according to Binder et al. [166] and again pure Ti coating on Ti6Al4V adhesion strength was determined with a tensile test by Price et al. [50] being from 32 MPa to 37 MPa. Adhesion of TiO_2 on various substrate materials has been studied by Tjitra Salim et al. [25]. Cold sprayed TiO_2 coatings were successfully deposited onto various substrate materials, which were inferred to be due to establishment of chemical bonding, which interestingly contradicts with the mechanism suggested by Kliemann et al. [76]. Moreover, a link between substrate hardness and adhesion strength was obvious: the harder the substrate the weaker the interfacial bonding, and thus, the weaker the adhesion strength. The overall values of adhesion strength, however, remained under 2 MPa. [25] The adhesion and cohesion strengths of the cold sprayed coatings relevant for biomedical applications are gathered up in the Table 5.4.

Table 5.4. *Sum up table over adhesion or cohesion strengths of the cold sprayed coatings.*

coating material	substrate material	author	spraying temperature (°C)	spraying pressure (bar)	shield gas	porosity content (%)	shear adhesion strength (MPa)	tensile adhesion strength (MPa)	failure type
Titania (TiO_2)	Aluminium, Titanium, Steel, Copper	Tjitra Salim et al. [25]	500	30	N_2	N/A		0,6 - 1,6	N/A
Magnesium	Stainless steel, Aluminium	Suo et al. [65][66]	500	25	Air/Ar	1,0 - 3,5		2,2 - 3,3	adhesive
Al-Al2O3	Magnesium	Wang et al. [64]	125	6	He	1,5	44		adhesive / cohesive
HA	PEEK	Lee et al. [20]	400	14	Air	N/A		7,16	substrate
Titanium	Aluminium	Binder et al. [166]	1000	40	N_2	0,13	81		cohesive
Titanium	Ti6Al4V alloy	Price et al. [50]	25	29	He	N/A		32 - 37	resin
Titanium (Mg porogen removed)	Titanium	Sun et al. [165]	340	10	He/N_2	48,7		42	resin
Titanium / HA (Al porogen removed)	Titanium	Qiu et al. [164]	370	7	He	60 - 65	20		N/A
Titanium / HA	Aluminium	Choudhuri et al. [162]	600	35	N_2	very low	24		cohesive

At present, very little information is available regarding cold spraying of unconventional metal alloys except Ti alloys. In contrast to conventional metallic biomaterials, the adhesion strength of biodegradable alloys such as Mg, is not necessarily a matter of long-term stability. In a study conducted by Suo et al. [65] adhesion strengths of 3,3 ± 0,8 MPa and 2,2 ± 0,8 MPa were measured using a pull-off test for cold sprayed Mg on Al and stainless steel plates

respectively. Furthermore, greater degree of mechanical interlocking associated with embedment of Mg particles was deduced as the main reason for more intimate bonding with Al. Furthermore, an adhesion strength several orders of magnitude higher were obtained by means of substrate heating [66]. Another examination was made by Wang et al. [64] whereby the adhesion was evaluated for Al-Al_2O_3 coatings cold sprayed on Mg substrate and revealed maximum shear stress peak of around 40 MPa with coating composition of 50 wt% Al – 50 wt% Al_2O_3.

Unlike with metals, the mechanical interlocking is mainly responsible for adhesion of cold sprayed particles onto polymer substrates. [19] [30] [31] [33] [105] [167] Intimate bonding was reported by Burlacov et al. [167] with ceramic TiO_2 particles deposited by cold spray on PS substrate. The reason for well-adhered coating structure was revealed as described by Figure 5.15. However, numerical data concerning adhesion strength of such coatings is limited. As an exception Lee et al. [20] has recorded adhesion strength as high as 7,16 MPa for HA coating deposited onto PEEK disk by cold spray. Unexpectedly, the failure took place in the substrate matrix while the coating itself remained intact.

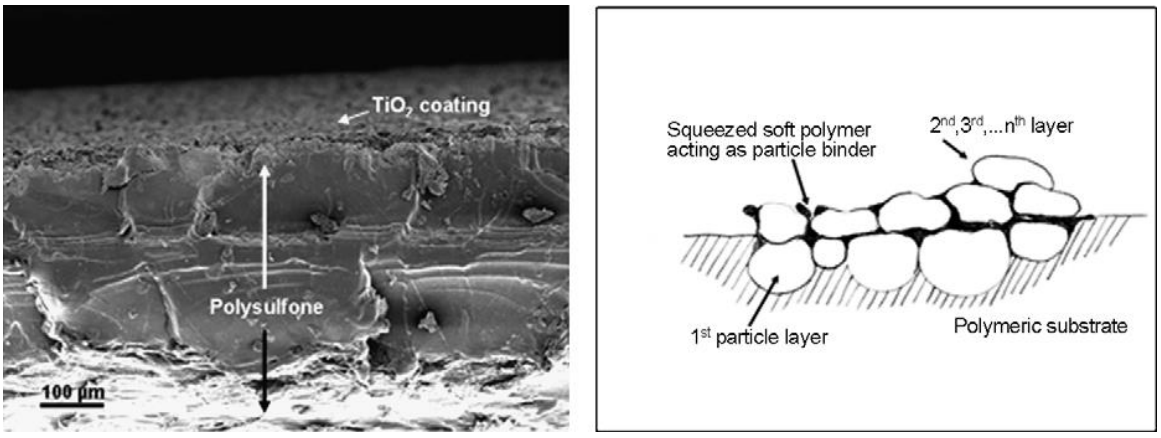


Figure 5.15. *Interlocked TiO_2 particles in PS matrix. The embedded layer of particles enable the accumulation of the following particle layers by squeezing out polymer ridges that act as binding matrix [167].*

5.2.4. Elastic (Young's) modulus and hardness

Stress shielding is a major complication involved in loosening of an implant when the uniformity of bone-implant interface is lost. It is attributed to heterogeneous distribution of biomechanical forces arising from mismatch of the elastic moduli of natural bone and artificial prosthesis as was indicated in Table 5.3. In other words, an incomplete interplay between osteocytes and the implant results in lack of stimulus for bone cells to sustain sufficient bone mineralisation, which is expressed by bone atrophy over time as documented in Figure 5.16. [128] [168]

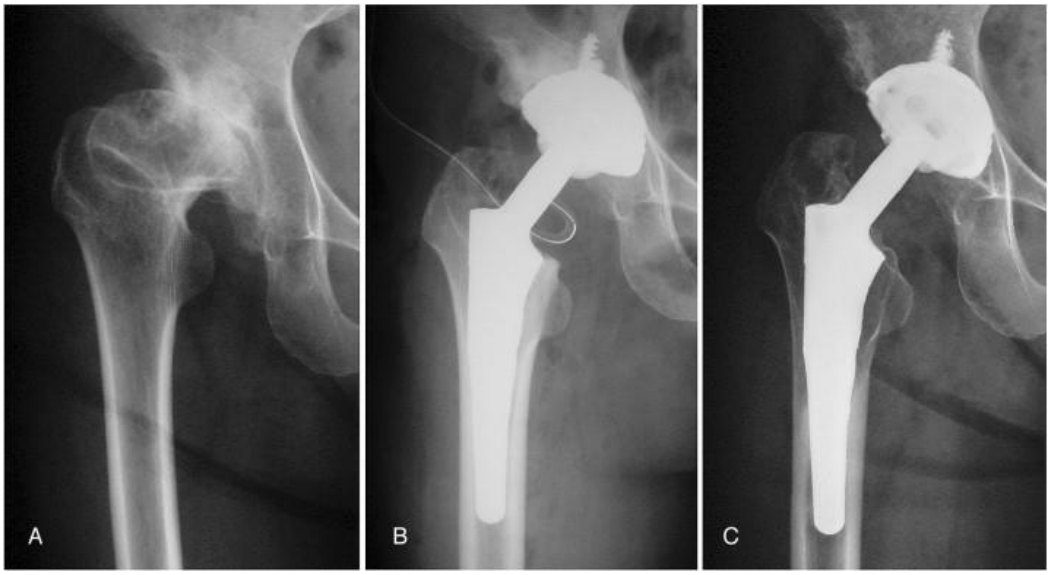


Figure 5.16. *Bone atrophy in the upper part of the femur as a result of stress shielding effect. Radiograph taken preoperatively (A) and immediately after operation (B) show initial bone density. Lower bone density is clearly seen in image taken 6,5 years postoperatively (C) [169].*

In Figure 5.16. deterioration of bone tissue is clearly visible in the upper part of the femur revealed by higher transparency for x-rays of femur in image C compared to image B. However, the underlying cause beyond this macroscopic process of stress shielding is at the micro-level: a biomechanical environment enables ideal cell proliferation relayed by integrin activation only when the elastic modulus is within a well-defined window. [128] [170] [171] At present, efforts are being made in order to overcome stress shielding effect induced by mechanical isolation: Cuts in values of elastic modulus are being pursued with metallic materials whereas the polymers, such as PEEK, and fiber-reinforced composites are seen as an increasingly attractive alternative meanwhile. [44] [172] [173] [174] [175] [176]

From the perspective of cold spraying, elastic modulus is an aspect of consideration, and particularly, the effect of deposited coating on elastic properties of the coated component is of a great interest. Moreover a mismatch between the elastic moduli of coating and of substrate has implications far beyond the altered overall stiffness: it contributes some forms of wearing and fatigue such as spallation. [78]

In the studies by Sun et al. [165] for a porous Ti coating obtained with vacuum sintering accompanied cold spraying, a compressive elastic modulus of 4 GPa and bending elastic modulus of 14.5 GPa were presented. Cizek et al. [27] found elastic modulus value of 36.7 GPa for a cold sprayed Ti coating superior to corresponding value of 7.2 GPa for plasma sprayed counterpart, which reflects remarkably higher porosity content. Similarly, elastic modulus of 20 GPa was measured under compression for cold sprayed Ti specimens by Price

et al. [50], by whom a conclusion was drawn that poor interparticle bonding was responsible for the elastic modulus of only a fraction compared to five-fold higher modulus of bulk Ti.

For cold sprayed HA coating on Mg substrate the elastic modulus was found to be around 9 GPa according to Noorakma et al. [62] maximum values measured at the top layer. A significant dissolution-dependent drop is typically observed in elastic modulus of HA coatings within a few weeks after an implantation. [56] Another osteoinductive material, suspension plasma sprayed bioactive glass prepared by Cattini et al. [98], possessed an elastic modulus ranging from 15 to 24 GPa.

Raj et al. [177] found consistent increase in elastic modulus of vacuum plasma and cold sprayed Cu alloy coatings. Instead, the determined Young's modulus was considerably lower for cold sprayed coatings in as-sprayed condition than for coatings which were subjected to annealing post-treatment. For plasma-sprayed TiO_2 and HVOF-sprayed nano-structured TiO_2 an improvements of 10 % and 23 %, respectively, were registered by Ibrahim et al. [78] These results were inferred to be due to employed nano-size particles and, conflicting with the findings of Raj et al. [177], higher degree of cold-working attained with HVOF technique. However, the elastic modulus of a thermal spray coating is also affected by porosity content as evidenced by Schrooten et al. [178] who demonstrated the impact of porosity content of a coating consisted of bioactive glass on elastic modulus. As opposite, in studies conducted by Kang et al. [23] and Chen et al. [179] the structure of a cold sprayed coating was successfully modified in adding reinforcing carbon nanotube –particles.

The results obtained by Bolelli et al. [72] revealed an elastic modulus values for cold sprayed Ta of slightly below 200 GPa, which are comparable to bulk material. By contrast, Cu particles embedded into various polymer matrices did not increase the elastic modulus considerably as hypothesized by King et al. [31] and remained below 2.5 GPa with PA6, PU, HDPE, PTFE, PP, and PC.

5.3. Phenomena detrimental to implant performance

Corrosion and wearing might be hazardous to mechanical properties such as fatigue endurance and adhesion strength. Moreover, lowered mechanical properties might lead to a sudden implant failure. Alternatively, biocompatibility might be degraded as a consequence of excess of corrosion products or wear particles leading to an activation of defence mechanisms.

5.3.1. Corrosion, dissolution and degradability

The aqueous environment with high quantities of dissolved ions and molecules within the human body provides an excellent foundation for extensive corrosion, dissolution, and degradation, to occur. As a distinction to many extracorporeal applications, these events must be carefully controlled since the occurrence of complications increases along with the amount of dissolved elements. Apart from this, corrosion has contribution to corruption of mechanical properties with fatigue and wear, which typically co-exist [153]. Therefore coating technologies are exploited to produce controllable prostheses with varying degree of corrosion protection or varying rate of resorbability. [81] [180]

Corrosion is a detrimental phenomenon that takes place at metal surfaces as a result of reduction-oxidation reactions which are driven by a difference of the electrochemical potentials between two separate metallic areas. The entire process is facilitated by ion-containing fluid that mediates the migratory ions that participate the reduction reaction while the metallic ions gradually dissolve into the fluid. Also an electric contact is required for transferring electrons from anode to cathode. However, with many metallic materials the corrosion current unexpectedly remains well below the values estimated solely on the basis of potential difference and hence, another conceivable event, passivation needs to be addressed. During passivation certain metallic element reacts with the oxygen to establish an oxide layer on the top of the metal, which acts as a barrier between the metal substrate and its surroundings. Therefore, as the amount of corrosive interactions is restrained by the limited rate of diffusion of the metal atoms through the passive film corroded areas are rarely identified on alloys containing Cr, Ti, Al, etc., which display a spontaneously-formed oxide layer. [181] As a consequence, in terms of corrosion these elements are essential ingredients of conventional biomedical alloys such as stainless steel, CoCrMo, and Ti6Al4V. The relative electrochemical potentials i.e. susceptibility to corrosion of biomedically relevant metals are presented as a galvanic series in the Table 5.5.

Table 5.5. *Galvanic series in seawater. Compiled from [182] [183].*

most anodic/ most susceptible to corrosion
magnesium
zinc
aluminium
carbon/ low-alloy steel
gray and ductile cast iron
304/ 316 stainless steel (active)
nickel (active)
chromium
tantalum
copper
304/ 316 stainless steel (passive)
zirconium
titanium
platinum
most cathodic/ least susceptible to corrosion

From the biological point of view, dissolution of the metallic elements is an issue of a special concern irrespective of the devoid of large-scale scientific evidence directly linked to implants. Another reason might be the lack of knowledge about the mechanisms of action or accumulation of these corrosion products. Nevertheless, increases of several orders of magnitude in serum ion concentrations have been observed following the implantation. Especially elements with known cytotoxic effects such as Al, Cr, V, and Ni are considered avoidable. The symptoms are diverse and following has been reported with respect to corrosion products: alterations in reproduction system and neuronal system related with Cr^{4+}, correlation between administration of Co-ions and cardiotoxicity and DNA damage, Ni-ion –generated cyanosis, death, and DNA damage, and, cardiotoxicity and toxic effect on reproductive organs. In addition, at present, all of the elements predominantly used in biomedical prostheses hold a potential to generate secondary inflammatory response including titanium and many of the aforementioned harmful elements take part to oxidative reactions that lead to releasing of free-radicals, which ultimately oxidise the cell membranes when protection of antioxidants is weakened. Although the majority of the specific routes of action are still unveiled, some general effects have been elucidated. [184] [185] These matters might serve as motives beyond the transition from metallic implants towards polymeric substitutes even though polymeric endoprostheses are not free of limitations such as aqueous dissolution.

Corrosion might be divided into multiple sub-categories. Firstly, pitting corrosion is a localized type of corrosion typically triggered by chloride ions and most often attacks the surface of a stainless steel. The initiation of pitting occurs at a small domain subsequent to breakdown of a passive layer and progresses perpendicularly to surface plane forming a mono-tubular structure. For the systems dedicated to load bearing the localisation of the corrosion is particularly fatal because the mechanical properties are abruptly deteriorated by rapidly progressive pitting corrosion. Surprisingly, acceleration of meta-stable pitting has been reported at the surface of Ti implants in physiologic environment [81]. Another example of localised corrosion is crevice corrosion, which preferentially attacks small crevices and is responsible for elevated concentration of chemically aggressive substances at the vicinity of the corrosion site, which encourages further dissolution. Being relevant to many biomedical in vivo applications, crevice corrosion is a serious problem in multi-component systems wherein clefts/cleavages are evident. The third type of corrosion: fretting corrosion is induced by abrasive micro-motion, which constantly removes the self-protecting oxide layer. As a consequence, the condition of the surface continuously varies from activation to repassivation. Posterior to replacement operation, this type of corrosion is closely related to accumulation of wear debris and high concentration of metal ions within the joint capsules. Moreover, the endoprostheses are prone to galvanic corrosion, which is highly probable when a system consisting of dissimilar metals is introduced in vivo. The current between these two dissimilar regions is generated by the difference between their characteristic electric potentials and hence, galvanic corrosion is increasingly detrimental when the size of an anode is a fraction compared to that of cathode. [81] [180]

Most frequently, the approach of choice is to prevent corrosion by means of coatings, when the coating acts as a barrier that does not subtract the driving force but considerably limits the reaction rate the electrochemical equilibrium is to be reached. In terms of permeability, cold sprayed coatings exhibit dense, porosity-free structure, which may provide an impermeable barrier for external fluids and therefore, inhibit substrate corrosion. As an example, Ta coatings deposited by cold spray technique performed superior anti-corrosion capacity to the references coated with inert-atmosphere plasma spray according to Koivuluoto et al. [22] Investigations by Dzhurinskiy et al. [186] have revealed, that an addition of Al_2O_3 particles into a pure low-pressure cold sprayed Al correlated with intensified corrosion. Presumably, an analogous relation between the quantity of metal-oxides and the overall corrosion resistance might be found with other light alloys. Mixtures of stainless steel 316L powder with different proportions of Co-Cr particles were cold sprayed on a stainless steel substrate in a study by Al-Mangour et al. [40] whereby a consistent trend of decreasing corrosion rate was observed after an annealing heat-treatment carried out at 1100 ^{o}C irrespective of the composition of the coating. Post spray heat treatment was also accomplished with HA-Ti composite coatings by Zhou et al. [24] The potentiodynamic measurements for immersed specimen in the simulated body fluid

confirmed a remarkable enhancement in corrosion resistance. In a study conducted by Trentin et al. [26] an attempt was taken by a cold spray deposited Ti coating to preserve a CoCr substrate beneath. As an outcome, diminished corrosion-resistance was reported, which is congruous with the findings of Al-Mangour et al. [40] obtained with Co-Cr-stainless steel – coatings. Moreover, Ti coatings with dense and pure sublayer and porous outermost layer of TiO_2 were under investigations of Zhou et al. [39] whereby an outcome of slightly impaired corrosion resistance subsequent to cold spray deposition was evidenced by potentiodynamic and electrochemical impedance spectroscopy measurements. However, the successful implementation of post-deposition heat treatment restored the corrosion characteristics almost to the initial level. Considering the facts postulated above, only a few coatings have been prepared that satisfy the requirement of protection against corrosion. In order to modify the electrochemical behaviour, annealing post heat-treatment is an advantageous technique to deploy since the enhancements in corrosion properties were highlighted.

In contrast to corrosion protection, bioresorbable alloys such as Mg, being extremely susceptible to corrosion, have shown their potential for controllably decomposing implants. Noorakma et al. [62] examined the behaviour of Mg-based AZ51 alloy substrate coated with cold sprayed HA in simulated body fluid. Extensive Mg dissolution was concluded during the 14 days of immersion due to the following reaction.

$$Mg(s) + H_2O(aq) \leftrightarrow Mg(OH)_2(s) + H_2(g)$$

By contrast to many metallic materials known by the unacceptable effects related to their dissolution, Mg is known for its bioactivity as reviewed by Witte et al. [187] and has a positive effect on the regeneration of bone tissue. Up to the present, no other studies to our knowledge, excluding those of Suo et al. [65] [66] not involving corrosion studies, have been published on fabrication of cold sprayed coatings using biodegradable materials such as Fe alloys, amorphous calcium phosphates, or various polymers.

5.3.2. Wearing, lubrication and friction

Joint surfaces are highly susceptible to wearing, sometimes to the extent that the only choice is surgical intervention. The major challenges regarding the joint replacements are besides wearing itself, the long-term complications related to wear debris. Aseptic loosening has been reported as the major reason for post-implantation revision surgeries the percentage varying between 38 % and 70 % from all the revision cases involved [188]. Such degeneration of bone is catalysed by the presence of inflammatory substances which, in turn, originate from macrophage cells dedicated to eliminate the wear-induced particles, as described more in-depth in the chapter 5.1.4. [189]. Apart from stimulated osteolysis, the release of wear particles also gives rise to 3-particle wear and increase the concentration of dissolved ions

discussed in the previous chapter. Additionally, post-mortem studies have evidenced an accumulation of wear particles in internal organs. [180]

The natural environment within a synovial membrane comprises the highly elastic cartilages separated by a non-Newtonian, lubricative film. Until the film thickness remains clearly above the surface roughness, a complete lubrication is anticipated. The surface roughness of artificial joint surface might be three times smoother than that of the biological equivalent. However, in the biological conditions the dominance of elasto-hydrodynamic lubrication, i.e. significant elastic deformation displayed by the articular surfaces under compressive loading, and the presence of a lubricative fluid collectively explain for an excellent wear-resistance. [130] [190] Therefore, as the joint surfaces are lubricated by synovial fluid adhesive wearing is considered as the main wear mechanism on implant surface [47]. Other wearing phenomena commonly identified on the coated implant surfaces include fretting and delamination that is typically linked to breakdown of the oxide layer and corrosion.

Unfortunately, deploying conventional biomedical alloys in the load bearing endoprostheses often conflicts with demands set for joint surfaces. For example, low wear resistance associated with titanium and its alloys has been widely acknowledged [47] whereas applying stainless steel or CoCr –alloys might result in a release of carcinogenic ions with unknown consequence. For these reasons, in order to minimise the wear and friction, many of the joint prostheses take an advantage of UHMWPE paired with ceramic, metallic or polymeric counterface. The wear rate with such pairings might be over 100-fold greater than e.g., with Al_2O_3-Al_2O_3 pairing [81]. Therefore, relatively large quantity of wear debris originating from UHMWPE is produced provoking the cells to response. Though this accounts for the higher number of inflammations recorded with UHMWPE, there are some other concerns related to ceramic or metallic pairings such as the considerably smaller size of the released particles and their biological effects on periprosthetic tissue, which validate the use of UHMWPE. [189] [191] These effects were studied by Jiang et al. [192] in living bacterial cells and a dramatic difference in toxicity was found between bulk particles and nanoparticles. The mortality rates were significantly higher with all three types of bacteria exposed to Al_2O_3, SiO_2, and ZnO nanoparticles than to bulk particles, and essentially, the results were independent of ion concentration of the material in question. Consequently, the toxicity was inferred to solely result from particle size and class. Wear characteristics of some material pairing candidates suggested for total hip replacements as a function of particle size in the Figure 5.17. and wear rates in the Figure 5.18.

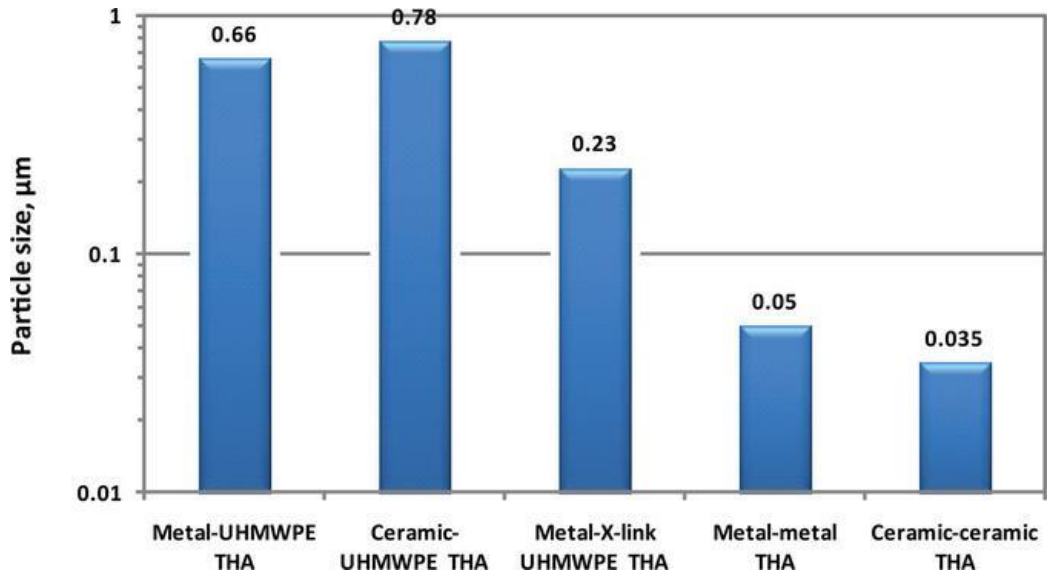


Figure 5.17. *Comparative diagram of particle size released from different material pairings used in total hip arthroplasty (THA) [193].*

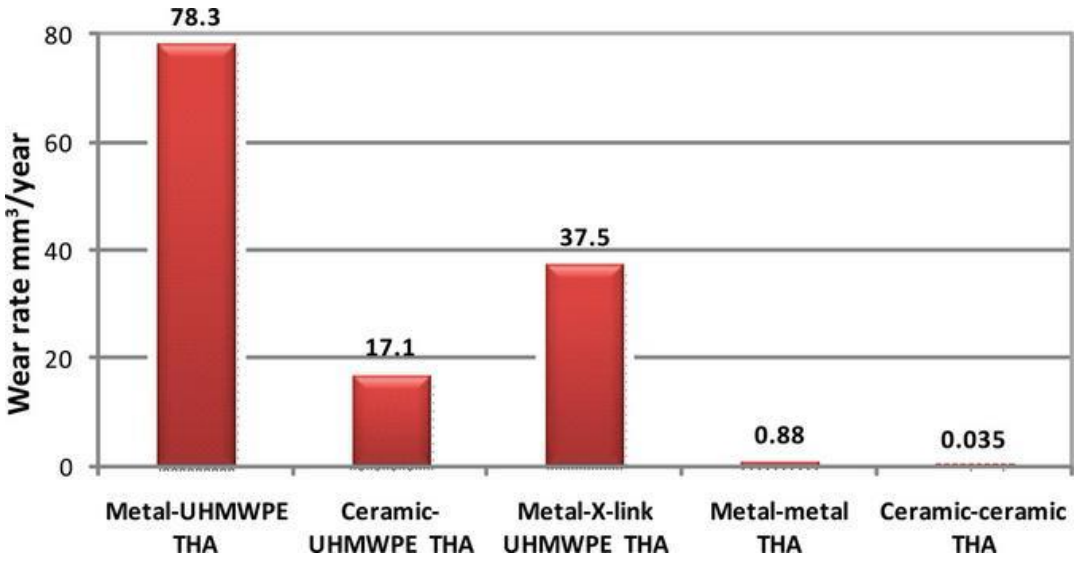


Figure 5.18. *Comparative diagram of wear rates for different material pairings used in total hip arthroplasty (THA) [193].*

There is a shortage of scientific papers that discuss the biotribology of coatings prepared by thermal spraying, and particularly concerning the cold spray technology, no relevant source is available. The sliding wear resistance of plasma sprayed bilayered ZrO_2/Al_2O_3-TiO_2 coating system in simulated body fluid (Hank's solution) was under investigations of Sathish et al. [194]. A lower sliding wear rate of $7.4 \cdot 10^{-9}$ mm^3/Nm with bilayered ZrO_2/Al_2O_3-TiO_2 was observed in contrast to wear rate of $3.7 \cdot 10^{-6}$ mm^3/Nm for plasma sprayed monolayer ZrO_2 and $1.48 \cdot 10^{-6}$ mm^3/Nm for monolayer Al_2O_3-TiO_2. Hence, wearing performance

superior to either monolayered ZrO_2 or Al_2O_3-TiO_2 coatings was inferred. Similarly, Yugeswaran et al. [57] documented an enhancement in sliding wear resistance of plasma sprayed HA coatings following an addition of YSZ. As an example for HA + 30 wt-% YSZ wear rate of $1.8 \cdot 10^{-5}$ mm^3/Nm was reported in contrast to $2.7 \cdot 10^{-5}$ mm^3/Nm recorded for pure HA under a load of 30 N. It was attributed to reduced porosity and increased hardness. Apart from implants designed for joint surfaces, an abrasive wear is a meaningful phenomenon that occurs of temporary manner during the contact of the bony tissue and the biomaterial surface during a surgical insertion. This has been the premise of the abrasion wear tests conducted by Morks et al. [195] who explored the effect of incorporating 15wt-% SiO_2 and 10wt-% Ti particles in HA plasma sprayed coatings on wear properties. The results suggested a duplication of wear resistance in comparison with pure HA. Likewise, remarkable enhancements in wear resistance have been attained by researchers such as Chen et al. [179] and Kang et al. [23] who have taken an approach to include carbon nanotubes into Al coating structure by cold spraying. The former confirmed an increase of 40% in wear resistance corresponding to 1 wt-% addition of carbon nanotubes whereas, according to Kang et al. [23], the effect was even more pronounced with wear loss declining from almost 1.0 mg/s for pure Al 1050 alloy to around 0.1 mg/s for Al 1050 alloy reinforced with 1wt-% of carbon nanotubes.

5.4. Biocompatibility and functional properties of implant surfaces

The principles beyond the concept of biocompatibility were elaborated in chapter 5.1. As a revision, a rough classification of biomaterials according to their level of biocompatibility is presented below in Table 5.6.

Table 5.6. *Classification of biomaterials according to biocompatibility as adapted from ASM International [196].*

Level of biocompatibility	Cellular response
Toxic	necrosis, apoptosis
inert	formation of fibrous tissue
bioactive	coherent cell attachment
bioresorbable	material replaced by tissue

In context of biomaterials, the biocompatibility is usually tested in two different level: in vitro and in vivo. Typically, the in vitro testing comprises cultivating of living cells for a few weeks in the laboratory conditions on the biomaterial surface accompanied by evaluation of the cell response throughout the surface in terms of cell proliferation, cell adhesion, cell size

and shape, and cell deaths. Thereafter, the biomaterials with the most potent performance in vitro might be chosen to proceed with to the stage of in vivo testing. Undertaking these clinical tests, the biomaterials are implanted and their biological response is followed up for months or years. These tests play a key role in verifying the in vitro results and predicting the later success. Sometimes, instead of cultivating any living organisms on the coating surface, a simple approach of in vitro testing is preferred to immerse the specimen in a solution called simulated body fluid, which composition was presented in Table 5.1. and is abbreviated as SBF. There are two commonly used, alternative solutions: Ringer's solutions and Hank's solution, with only subtle compositional differences. [147] This chapter discusses the features of a biomaterial surface that dictate the cellular response, and how the present advances in the field of cold spraying fulfil these material requirements.

5.4.1. Enhancement in biocompatibility by biomaterials

The type of material plays a fundamental role in terms of biocompatibility. The metal surfaces were recently the subject of in vitro study by Hofstetter et al. [197] with a number of different gene expression indicators involved. Consequently, a higher degree of cell viability and osseointegration was facilitated by Ta and Nb surfaces than Ti or Zr surfaces. [197] Among the metals, for example, the biological response has been evaluated based on in vitro and in vivo investigations accomplished by Tang et al. [68] with plasma sprayed Ta coatings. Considerably higher survival, adhesion and proliferation of human bone marrow stromal cells were observed on Ta than on Ti coating manifested by the prevalence of the osteoblast differentiation-related markers alkaline phosphatase (ALP) and Runt-related transcription factor 2 (RUNX2) diagnosed by fluorescence detection. Good osseoconduction was attributed to i.a. rapidly passivating surface oxide layer. In the same study, an in vivo trial was taken as a rabbit femur implant model was applied to plasma sprayed Ta and Ti implants wherein the higher rate of osteogenesis was affirmed on Ta. [68]

In depositing HA layer on PEEK substrate by cold spray technique, Lee et al. [20] acquired a substantial enhancement in osseoconductivity comparing to bare PEEK surface: In vitro tests were conducted with human bone marrow mesenchymal stem cells. The reasons stated for superior cell attachment and proliferation onto HA than PEEK substrate included highly wettable, hydrophilic, and rougher surface. In vivo results showed consistent promotion in biocompatibility. Likewise, enhanced osteogenic ability has been documented on plasma sprayed HA coating on PA as well as Ti substrates. Proliferation of human fetal osteoblast cells and the presence of osteoids was observed on the HA surface, which were absent on the pure Ti according to Roy et al. [198]. Deposited onto PA-based composite substrate, HA coating induced a bone-like apatite layer during a 28-day-immersion in SBF as demonstrated by Auclair-Daigle et al. [112] Additionally, the good cell viability of the plasma sprayed HA has been affirmed in vivo by Hacking et al. [113] A comparative study performed by Legoux

et al. [80] for plasma sprayed HA on PA substrate and HVOF-sprayed HA on Ti-6Al-4V substrate revealed a slight enhancement in cell adhesion and proliferation of the polymer substrate comparing to Ti substrate. On the other hand, the highest activity of cultured osteoblast cells was found on the surface of HVOF-sprayed nano-TiO_2 coating, which also was involved in the study. In addition, the osteoblasts had essentially flattened appearance on nano-Ti whereas inhabiting the HA surface an elongated shape is usual. Similarly, the HVOF-sprayed nano-TiO_2 coatings investigated by Lima et al. [199] induced more favourable cellular (human mesenchymal stem cells) responses than commercially dominating plasma sprayed HA as shown by the number of cultivated human Mesechymal Stem Cells in Figure 5.19. Li et al. [200] reported that a marked improvement in osteoblast attachment and proliferation was obtained in spraying HA coating on the surface of Ti substrate by plasma- and HVOF spraying. At the same time the critical role of HA phases was emphasised with respect to osseoconduction, which outweighs the effect of nanostructure though it has a contribution. An innovative approach was adopted by Qiu et al. [164] to produce a porous composite coating consisting of HA and Ti by cold spray technique. In vitro tests were carried out in simulated body fluid and in a culture of human osteosarcoma-derived cells. However, despite of the fact, that the tested specimen showed high affinity for Ca^{2+} mineralisation in SBF conditioning, the sovereign biocompatibility in terms of metabolic activity of the osteoblasts was detected at the surface of $Ca_3(PO_4)_2$ specimen, which was used as a reference.

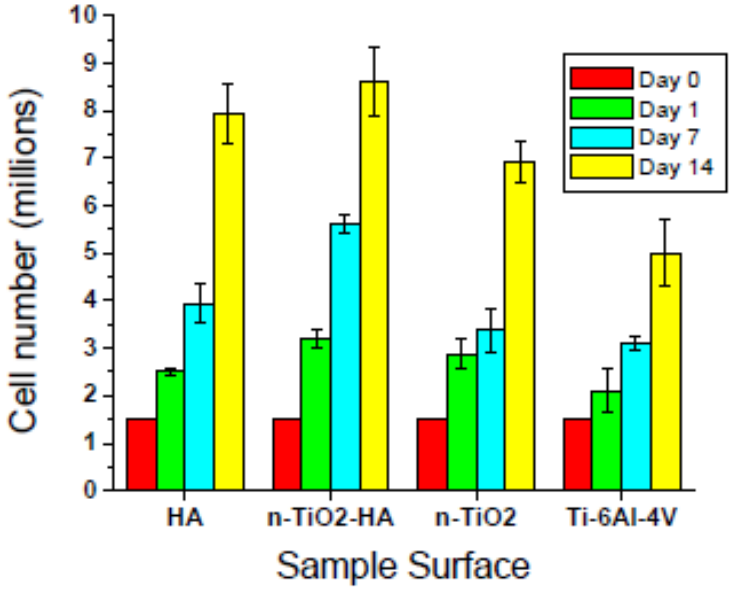


Figure 5.19. *human Mesechymal Stem Cell proliferation on plasma sprayed HA, HVOF-sprayed nano-TiO_2-HA, HVOF-sprayed TiO_2, and bare Ti-6Al-4V measured using Alamar Bleu –indicator. [199]*

Biocompatibility of different types of bioactive glass has been the subject of investigations. An extensive in vitro study was published on plasma sprayed $Ca_2ZnSi_2O_7$ and $CaTiSiO_5$ by Wang et al. [99] Acellular mineralisation as well as primary human osteoblast proliferation tests were performed for the specimen both of which suggested for preferable biocompatibility of $Ca_2ZnSi_2O_7$ which was believed to be due to Zn^{2+} ion release. It was also pointed out, that both bioactive coatings gave rise to emergence of bone-related proteins RUNX2, osteopontin and type I collagen in comparison with bare Ti-6Al-4V alloy. Depositing bioactive glass in suspension has been experimented with high velocity suspension flame spraying (HVSFS) by Bolelli et al. [93] in employing SiO_2–P_2O_5–CaO–K_2O system and with suspension plasma spraying by Cattini et al. [98] in employing Na_2O–K_2O–CaO–MgO–P_2O_5–SiO_2. Both of these in vitro studies followed identical testing procedure, wherein the specimen were immersed and the conclusion was drawn in the basis of analysing the mineralised structure on the top of the coating. The results indicated gradual development of carbonated HA as a surface layer with mechanism identical to bulk bioactive glass.

Coatings made of HA with varying amounts of YSZ (0 – 30 wt-%) were prepared by plasma spraying by Yugeswaran et al. [57] and their biocompatibility was firstly examined in SBF solution, which was thereafter accompanied by culturing marrow stromal cells on them. Ambivalently, HA formation was detected in all coating surfaces except the one containing 10 wt-% YSZ. As to succeeding cytocompatibility test, no sign of cytotoxicity was shown. In another study of Wang et al. [83] plasma spraying was utilised in order to create nanostructured 3Y-TZP coating. In vitro immersion in SBF resulted in precipitation of HA on as-sprayed coating whereas being absent on the corresponding bulk surface. Such behaviour was deduced to owe to its nanostructure as well as established Zr-OH-groups. Good cell viability with complete coverage of the coating surface was attained within 7 days.

As to bioresorbable polymeric materials, cell proliferation of human osteoblasts on PHBV/PMMA was, according to Chebbi et al. [115], almost identical to that on bare Ti substrate irrespective of the polymers having exposed to extreme temperatures during flame spray process. As to cold spraying, limited number of studies are available that deal with in vitro cell viability testing.

5.4.2. Macroporosity and tissue ingrowth

Tissue ingrowth, being one of the principal concepts closely related to biocompatibility, is defined as an ability of tissue to grow inside the biomaterial to the surface asperities. Addressing boneous tissue, by growing within the surface voids, strong interlock is established between an endoprosthesis and bone as depicted in Figure 5.20. It is widely accepted, that in order for tissue ingrowth and vascularisation to occur an open porosity ranging from 100 to 400 µm in size is required. [201] However, formation of osteoids have

been found in the pores down to 40 µm, but the smaller ones are typically filled with fibrous tissue instead of bone. [202] Clinically the extent of bone ingrowth is also governed by factors such as interfacial micromotion as well as interfacial distance [201]

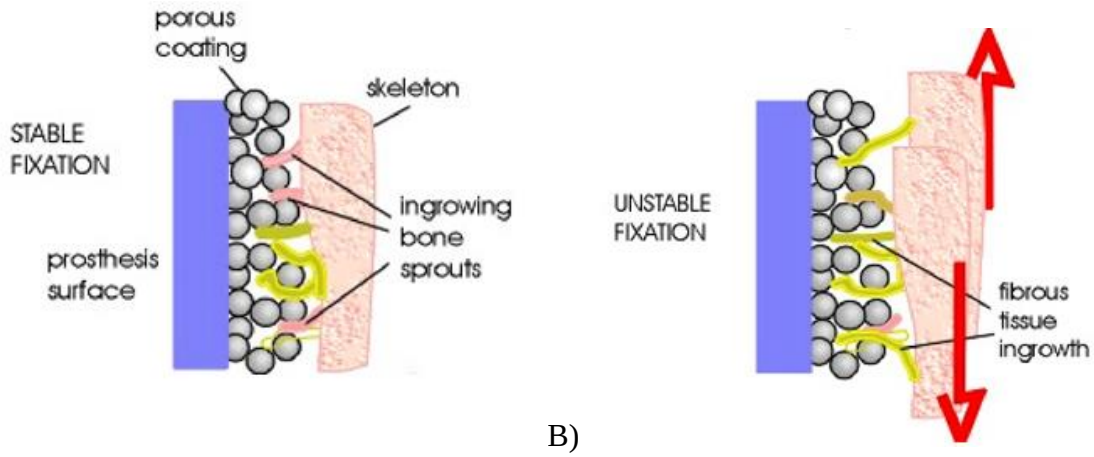


Figure 5.20. *A) Stable implant fixation by bone ingrowth into the pores of the coating. B) Unstable fixation caused by fibrous tissue ingrowth. Modified from [203].*

Using thermal spray methods, coatings with known porosity of 10 to 20 % are easily attainable, and adjusting the spraying parameters, coatings with very high level of porosity might be fabricated. However, the mechanical properties are unavoidably compromised when such high porosity structures are produced. With this respect, the tamping effect inherent to high velocity cold spray process might be beneficial in creating high porosity gradient structure illustrated in Figure 5.21. Successful deposition of such gradient porosity coating by cold spraying of sponge powder has been demonstrated by Choudhuri et al. [162] with composite structure consisting of Ti and HA. Gradient structure was attributed to cold spray tamping effect.

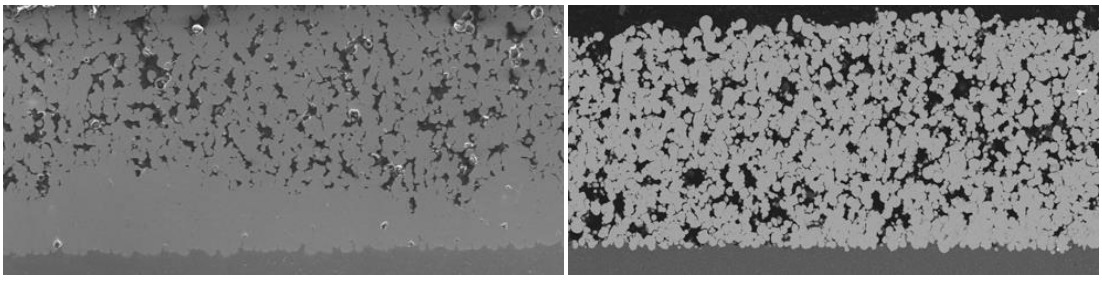


Figure 5.21. *Porous structure of a cold sprayed Cp-Ti coating. The porosity gradient is seen in the structure at the left (applied process condition 35 bar, 500 ºC, 400 m/s). The right-hand image holds a higher degree of porosity (applied process condition 25 bar, 500 ºC, 400 m/s) [162].*

The level of porosity was highly dependent on spraying parameters as indicated in Figure 5.21. Experimented by Qiu et al. [164] similar materials were innovatively mixed with 75 – 105 µm Al particles and propelled onto Ti substrate. Thereafter, the Al porogen was removed by alkaline solution. The remaining coating possessed a porosity of 60 to 65 % within the size range of 50 - 150 µm. An approach comparable to this was taken by Sun et al. [165] wherein 63 – 73 µm Mg particles were added on Ti powder and cold sprayed. Mg was removed by evaporation over the vacuum sintering. As a result, porosity of almost 50 % with sizes distributed between 70 and 150 µm was affirmed. The mechanical properties, such as bond strength measured by tensile adhesion test, remained surprisingly high. Yang et al. [204] experimented the porosity formation of Ti-48Al-6Nb coating under reactive sintering treatment preceded by cold spray deposition. The porosity content of 20 % with narrow pore distribution with an average of as low as 1,8 µm.

5.4.3. Surface roughness and topography

One of the primary interests with biomedical coatings is to create intimate bonding between a cell and an implant which, as mentioned earlier, is closely related to protein adhesion. Among the key factors affecting the protein adsorption and cell adhesion, are surface roughness and topography. As an attempt to enhance the interfacial attunement nano-structured surface topographies have been recognised as an effective tool to mimic the biological extracellular matrices [205] [206]. However, many of the techniques dedicated to produce such nano-topographies are complicated [206] [207]. Interestingly, increased proliferation has also been shown on nano-size materials or grains by many investigations. Although the enhanced protein adsorption to some extent, is caused by an increase in surface area it does not explain the improved cell adhesion alone when using nano-size materials instead of conventional micron-size materials. Rather, similar size of the details of the nano-structured surfaces and extracellular proteins has often been proposed to be the reason for priviledged protein adsorption on nano-surfaces. However, as such events are partly considered arbitrary of nature, experimental approaches have been popular. Based on experimental investigations Table 5.7. sums up the cell densities measured on the most common biomaterials. For cell attachment to occur an optimal spacing of 15 nm for the TiO_2 nanotube walls was proposed. Increased cell adhesion, proliferation, and osteoganic differentiation has been reported for particle arrays with a similar distance, which is probably due to ease of extracellular protein clustering when protein size corresponds to the spacing as suggested with integrin binding on TiO_2 nanotubes with 15 nm diameter. [128] [180]

Table 5.7. *Correlation between grain size and osteoblast cell density for most common biomaterials. Modified from [180].*

Conventional material	Cell density (cells/cm^2)		Nanoscale material
Titanium	1400	2000	Titanium
Ti-6Al-4V	950	1600	Ti-6Al-4V
Co-Cr-Mo	600	1450	Co-Cr-Mo
Alumina (167 nm)	5000	6000	Alumina (24 nm)
Titania (4520 nm)	7000	8000	Titania (39 nm)
Hydroxyapatite (179 nm)	7000	9500	Hydroxyapatite (67 nm)

Correlation between surface topography and cell viability has clearly been shown by many studies including those dealing with gold (Au) nanodot and TiO_2-arrays. For Au nanodots, spacing of 28 nm and 58 nm was preferred over 73 nm or 108 nm in terms of quantities of actin, FAK, and integrins on the surface. Actually, a slight decrease was observed in density of MC3T3-osteoblasts with nanodot array with 28 nm spacing comparing to nanodot array with 58 nm spacing. Similarly with TiO_2 -array, adhesion, proliferation, cell motility and differentiation of osteogenic cells was pronounced on the 15 nm nanotubes in comparison with a smooth surface or 50 – 100 nm nanotubes. [128] However, an investigation by Zhao et al. [208] revealed a crucial role of not only submicron surface structure but also micron-scale roughness for osteoblastic response. In their study an extensive differentiation of human osteoblast-like MG63 cells was observed with stimulated local factor production, which was attributed to surface roughened by sandblasting/acid and etching. [208] Nevertheless, some conflicting results have been presented, which highlight the complexity, unpredictable nature, and poor level of understanding of protein adsorption and cell attachment.

Gross et al. [209] surveyed the surface topography of various implants coated with thermally sprayed HA whereby the effects of surface topography on osteoclast reproduction were clearly indicated: refined surface structure correlated with cell viability, which was manifested by osteoclast resorption 10 times greater on as-sprayed coatings comparing to polished surface. With thermal spray coatings the splat shape and topography might be modified mainly by controlling the particle velocity and spraying temperature. Melting/solidification processes which, due to solid state characteristic are not relevant with the cold spray technique, are the important variables determining the surface topography. Nanostructured TiO_2 coating was prepared with HVOF-process by Lima et al. [52], who found an interrelation between the obtained nanotextured surface and incompletely molten particles whereas molten particles preferentially constituted smooth surfaces. Nanostructure of the TiO_2 was also deduced to be a reason for enhanced proliferation of osteoblasts in an in vitro study by Lima et al. [199] Owing to absence of high temperature phase transformations

during the deposition, these findings suggest that the initial shape and size of the powder particles might presumably have a great influence on the topography of cold sprayed coatings. Smallest topographical features of 40 – 60 nm with some agglomeration identified on the coating surface was reported by Noorakma et al. [62] with HA coatings prepared by cold spray as presented by Figure 5.22.

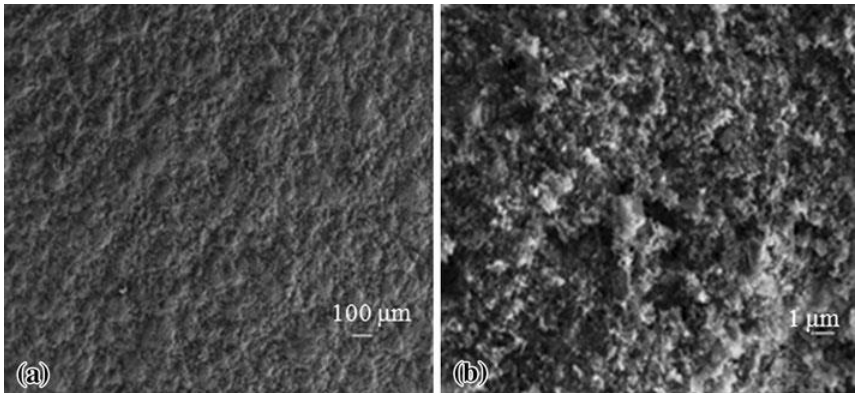


Figure 5.22. *Surface topography of the cold sprayed HA coating [62].*

Bae et al. [210] studied the formation of nanostructure prepared by cold spray from Ti powder. Plasma-atomised cp-Ti powder (mean size 22 µm) yielded a structure that varied from the dislocation-free grains smaller than 100 nm to the dislocation-rich grains over 250 nm. Moreover, according to Bae et al. [210] nanocrystalline (20 – 50 nm) structures have been successfully produced using Al and Ni feedstocks by Ajdelsztajn et al. [211] [212], but not with Ti. Very recently, HA was used in conjuction with graphene particles to fabricate a bone-inducive coating with nanotopography by Liu et al [92]. The structure of the feedstock particles (length 20 - 40 nm, diameter ~10 nm) produced by wet chemical reaction was well inherited by the coating deposited by vacuum cold spray method as depicted in Figure 5.23.

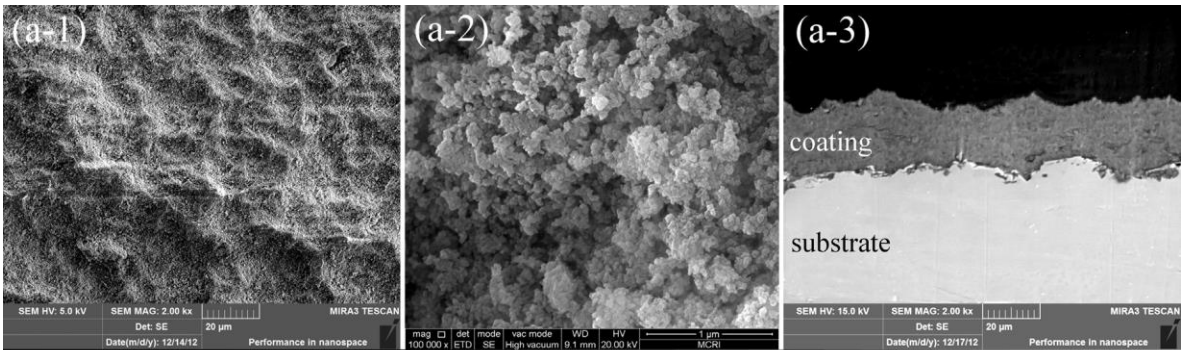


Figure 5.23. *Surface structure of a vacuum cold sprayed HA-graphene coating(a-1) and (a-2). Cross-sectional view of the coating structure (a-3). The scale of the image in the middle is 1µm as noted down in the image [92].*

Excellent results were obtained with these coatings regarding not only adhesion strength, but also cell proliferation as well as fibronectin-protein attachment onto graphene particles was promising. Furthermore, as to cold spray technique the surface structure of the coatings might presumably be modified with a simple blasting post-treatment. However, an attempt was made by Mustafa et al. [213] to blast a bulk Ti surface using TiO_2 particles sized from 63 µm to 300 µm as a blasting media, but no explicit or dramatic results was obtained in terms of cell metabolism and cell attachment suggesting, that the treatment was ineffective probably due to coarse, micron-size surface topography.

In terms of surface roughness, which has an impact on contacting area between the cell and the surface, following results have been recorded: Burlacov et al. [167] reported arithmetic average surface roughness of 1,8 µm and 3,4 µm was measured on TiO_2 coating cold sprayed respectively, at 450 ºC and 500 ºC on PS substrate. Such surface roughness R_a values are usual for cold and thermal spray coating, and are normally dictated particularly by particle size and spraying distance [209]. Likewise, cold spray method was applied by Shtansky et al. [214] whereby a variety of surface roughnesses from 4 µm to 80 µm was determined for a Ti coating. With thermal spray coatings, R_a values varied between 1,53 – 4,4 µm with PHB prepared with flame spray by Chebbi et al. [115]. As to Wu et al. [107] surface roughness of smaller than 5 µm was recorded on the plasma sprayed HA coating shown in a topographical Figure 5.24.

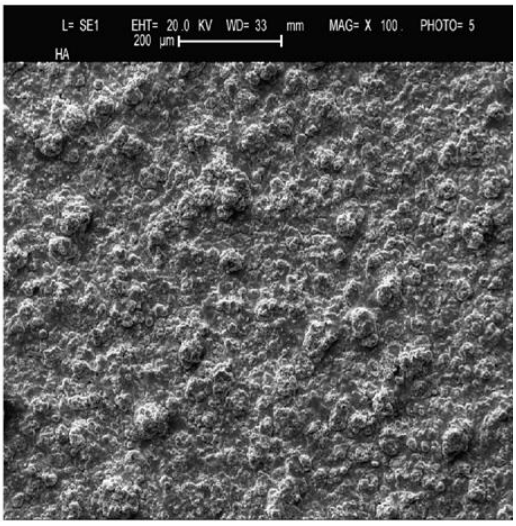


Figure 5.24. *Typical surface topography of a plasma sprayed HA coating with 4.9µm surface roughness [107].*

An opposite approach might be chosen when prevention of bacterial infections or soft tissue adhesion is desired. In such case tissue adhesion might be avoided in designing the surface topography with a deficient number of adhesion sites for extracellular proteins. Producing a smooth surface, might be a viable strategy to minimise the accumulation of the adhesive

proteins. [206] [215] An opposite strategy was chosen by Puckett et al. [216] wherein nanostructuring was used in order to suppress the bacterial activity.

5.4.4. Wettability and surface charge

Hydrophilic surfaces, defined by a contact angle smaller than 90º, are commonly considered more suitable substrates for triggering a favourable cellular response even though some counterarguments have been stated. However, the effect of wettability on cell functions is not easy to identify because the factors discussed above such as surface topography and chemistry all have a contribution to cell fate. [81] These factors along with varying protein-specific affinity of an implant surface account for the fact that in the molecular level, surface wetting properties are closely related to competitive protein adsorption phenomenon [138]. This has been appreciated by many including Hartvig et al. [134] who introduced a model to predict the adsorption behaviour of different types of proteins on the charged surfaces. The model was applied to explain the experimental results acquired with lysozyme and α-lactalbumin, and the dependence of the protein adsorption regulation on the factors such as pH, ionic strength and surface charge. Likewise, in investigations conducted by Yin et al. [217] on the adsorption of proteins on the HA surface, it was found to be governed by the pH, surface area and concentration of ions. Also, preferential adhesion of ions such as PO_4^{3-} and Ca^{2+} was hypothesised. A more extensive review on the surface charge and wetting behaviour of bioceramics has been published by Wang et al. [218]. The interplay of charged metal surface and adsorbable proteins has been demonstrated by Donatan et al. [219] who found altered amount and coverage of gold-binding peptide 3rGBP$_1$on Au surface as a response to a change in the surface charge. It was considered as an indication of the key role of surface charge in the protein adsorption phenomenon, and might be a viable tool to control the orientation of an individual peptide and the array of the proteins. In context of plasma sprayed coatings Xie et al. [220] recently reported superior cell response on TiO_2 nanotube layer that was produced by anodisation on plasma sprayed Ti coating in comparison with bare Ti coating. The enhanced cell response was linked to higher surface charge which had an accelerating effect on protein adsorption.

Chebbi et al. [115] found no significant alteration in wetting behaviour during thermal spraying of PHBV/PMMA polymer coatings compared to the values measured prior to deposition but instead, a correlation of impaired wettability with increasing coating thickness was observed. An average contact angle of 58,68 º was evidenced on the polymer coatings. In contrast, hydrophobic tendency was confirmed on Ti cold sprayed coatings realised by Shtansky et al. [214]: Contact angles varied from 99º ± 2º to 108º ± 4º as measured in as-sprayed condition. Nevertheless, post-treatments were carried out in order to overcome inferior wetting behaviour.

5.4.5. Inflammation suppression I: antibacterial particles

Up to this point our focus has been on promoting osseoinducive reactions. However, another aspect to consider is how to avoid unfavourable responses e.g., inflammations caused by bacteria by means of cold sprayed coatings. The bacteria frequently found at the implant surface comprise e.g., the most common contaminant of the metal surface *Staphylococcus aureus* and the most common contaminant of the polymer surface *Staphylococcus epidermidis* but numerous other pathogens exist such as *Escherichia Coli* and *Pseudomonas aeruginosa*. In preventing bacterial infections, the role of antibacterial coatings has been underpinned by the fact that an increasing number of stocks resistant to antibiotics have been recognised. [221] [74]

A number of approaches have been adopted to enhance the bactericidal capability of surfaces that come to a contact with the living tissue that is susceptible to inflammable pathogens. Therefore, in the field of cold spraying, anti-inflammatory particles such as CuO, Ag, MgO, and ZnO have gained a lot of attention and, at the same time, tremendously superior bactericidal effect has been linked to nano-sized powders in comparison with bulk material [192]. Some evidence has been presented suggesting that microbial cell membrane is the primary target of Ag^{+}-ions which, allowing the penetration of Ag^{+}-ions, results in disruption of the reaction chains of the vital metabolic enzymes. With solutions concentrated with MgO, an extensive damage in the cell membrane has been reported as well. [222] Considering CuO and ZnO, the electrostatic interactions of negatively charged cell membrane and positive charge of the eluted Cu^{2+} and Zn^{2+} -ions has offered an explanation for contact between the two. Subsequently, the ions tend to enter the cell and interact with the biological molecules e.g., Zn^{2+} with sulphur-containing sequences. [223] Hence, the killing ability of Cu particles is primarily defined in terms of concentration of the dissolved ions rather than the contacts of the antibacterial particles with bacteria. However, the accurate mechanisms remain unravelled. Additionally, the effectiveness of Cu and Ag particulates have been tested against viruses. The results have confirmed an antiviral capability of released Cu^{2+} and Ag^{+} -ions based on an elevation in pH and changes in conductivity. [222]

To date the majority of the experimental approaches have been concentrated on processing of Ag^{+}-particles because of the higher capability of these particles to eliminate pathogenic bacteria. Particularly active in the field of cold-spray technology have been Sanpo et al. [224] [225] [226] [227] by whom at least these four antibacterial studies have been released comprising an investigation wherein Ag powder was employed [227]. The capability of Ag particles was clearly indicated by the quantity of cultivated *E. Coli* on the coatings containing varying proportions of Ag. A coating with a composition of HA-Ag 20/PEEK 80 was ineffective to restrain bacterial proliferation with a multiplication of almost 100-fold comparing to initial state whereas a negligible increase in the quantity of *E. Coli* bacteria was

displayed by coating with the composition of HA-Ag 80/PEEK 20. Very similar bacterial growth rates to HA-Ag/PEEK coating were observed with Cu particles on a cold sprayed Chitosan-Cu/Al coating tested by N. Sanpo et al. [224]. An increase in amount of Cu particles correlated with antibacterial property and additionally, in bacterial culture a clearance zone surrounded the region that was covered by a Cu-rich coating. However, from the point of view of cytotoxical concerns Cu and Ag are not considered as the first line elements for biomedical in vivo applications and therefore, antibacterial elements such as Zn appears to be increasingly attractive. Antibacterial property of cold sprayed ZnO-Al coating experimented by Sanpo et al. [226] against *E. Coli* proliferation remarkably improved along with proportion of ZnO being four to five times smaller with a composition of Al20/ZnO80 comparing to pure Al. Another study by Sanpo et al. [225] carried out with cold sprayed ZnO/Ti coating suggested much higher antibacterial capability which unfortunately was accompanied by a drastic drop in osteoblast cell viability. In each study an agar plate cultivation of the bacterial cells was the method of choice, which was loyal to a testing procedure in which the extent of dissolution of antibacterial ions might not be equivalent to true exposure of the bacterial cells under in vivo environment. Antibacterial study conducted by Tamai et al. [228] with cold sprayed ZnO –glass (P_2O_5-SiO_2-Al_2O_3-CaO) composite coating displayed an antibacterial capability. 20 – 30 % ZnO –content was enough to diminish the number of *S. aureus* bacteria on the coating 20 000-fold and 15 000-fold with *P. aeruginosa* during the 24 hour test. However, it was relatively ineffective against *K. pneumoniae*. Apart from antibacterial applications, the toxicity of some elements to vertebral cells has been under investigation for antifouling applications such as Cu particles embedded into HDPE matrix by cold spraying [229]. However, as these surfaces display antibacterial property, they also might be relevant in the medical context.

Some interesting studies have recently been published regarding the antibacterial coatings prepared by using thermal spray techniques. Li et al. [223] found suppressed reproduction of *E. Coli* bacteria on a plasma sprayed $Ca_2ZnSi_2O_7$ coating due to release of Zn^{2+}-ions. The bactericidal capability was further enhanced by adding Ag particles into the coating [230]. Same sort of approach has been applied to realise composite coatings consisting of TiO_2-Ag powder that was plasma sprayed by Li et al. [231] and detonation sprayed by Dudina et al. [232]. In the former study it was shown that adding 5-wt% Ag particles in coating improved the antibacterial capability to the extent that colonies of *E. Coli* were not able to survive for 24 hours in incubation. Another important observation made by Li et al. was that no difference in osteoblast adhesion and proliferation was found between a pure TiO_2 and TiO_2-Ag over the course of the experiment. Likewise, according to Li et al. [233], Ag particles embedded into wollastonite ($CaSiO_3$) coating by plasma spray showed good capability against *E. coli* with killing ability of almost 100 % with Ag content of 1,63 wt-% within a day.

To our knowledge no scientific results are available based on in vivo examination, which would provide information about the clinical performance of the cold sprayed antibacterial coatings. Instead, there are investigations regarding the biological effects of thermally sprayed antibacterial particles, mainly Ag. Flame spraying was applied to fabricate a Ag-containing HA coating, which was subcutaneously inserted in rats in order to clarify the antibacterial behaviour against MRSA (methicillin resistant *Staphylococcus aureus*). A conclusion was drawn by Shimazaki et al. [234] that there is a transient peak in serum Ag^+ concentration within 72 hours after the implantation and an overall decrease in quantity of bacteria was clearly observed. Another study clinically dealing with the antibacterial efficacy of flame sprayed Ag-HA coatings was performed by Yonekura et al. [235] whereby serum Ag-ion level was followed up for a few weeks. With coatings containing 3 – 50 % Ag, the measured serum concentration of Ag^+ was 1,1 – 5,3 ppb respectively whereas the peak value obtained with 3-wt% Ag coating within 72 hours after insertion was 50 – 60 ppb according to the previous study. Antibacterial activity has been indicated with serum concentrations of 35 ppb and by contrast, adverse effects including leukopenia, hepatic and renal dysfunctions, and argyria have been reported with the Ag-ion content of as high as 300 ppb.

5.4.6. Inflammation suppression II: photocatalytic surface

Instead of simply fabricating coatings containing antibacterial particles that readily dissolve into an aqueous medium, another approach has been applied relying on a phenomenon called photocatalysis. The idea of photocatalysis is based on degradative redox reactions between catalytically active coating and an organic molecule in the presence of external UV radiation. At the centre of the scientific interest have been TiO_2 coatings, which during the past few years have been a subject of a couple of studies evaluating the applicability of cold sprayed coatings. With respect to photocatalytic TiO_2, the reason for emerging interest towards cold spraying as a technology is the possibility to avoid the transformational change from anatase to rutile, which ruins the photocatalytic capability. However, due to the requirement of external light source, the intended use of these coatings would most likely be on external medical devices and various other antifouling or sterile surfaces. Photoactive TiO_2 (anatase) coatings obtained by cold spraying were evaluated by Burlacov et al. [167] with an order-of-magnitude higher rate of 4-chlorophenol degradation and hence, photocatalytic efficiency, than the reference specimen soaked in equivalent solution. Interestingly, thermal degradation of the substrate polymer was mentioned as a considerable problem by the same study. Another study conducted by Yang et al. [236] a nanoporous 10 to 15 µm layer of anatase TiO_2 was successfully deposited using cold spray technology. In this paper, the photocatalytic efficiency was shown to be much higher with cold sprayed coating than with HVOF-sprayed coating primarily due to low processing temperature which preserves anatase structure, but also smaller particle size and greater surface area accounted for enhanced photocatalytic activity. According to Bannier et al. [73] harmful phase transformation also

occur in the coatings prepared by a novel suspension plasma spray technique. Most recently Ivanova et al. [237] demonstrated the catalytic efficiency of cold sprayed TiO_2 in degradation experiments which were performed using oxalic and dichloroacetic acids. The measured degradation rates for these substances during the exposure to UV radiation suggested an excellent overall photocatalytic activity which was superior with oxalic acid comparing to dichloroacetic acid.

5.4.7. Inflammation suppression III: anti-inflammatory agents

In a case of post-operationally inflamed tissue, at present, antibiotics are the first line treatment to suppress the inflammation and intravenous or oral routes have traditionally been used to administer these agents. In order to obtain a more intense and local effect at the site of inserted device the ongoing research is increasingly focused on developing implants with an immune evasive agent immobilised on the implant surface or impregnated in the implant matrix. Large number of such agents including glucocorticoids, anti-inflammatory cytokines, nitric oxide, etc., has been tested [144]. However, different approach might be chosen depending on the target application. For example, addressing the musculoskeletal implant, the anti-inflammatory agents are commonly delivered via bioactive calcium phosphates whereas the polymeric coatings are predominantly being utilised as a drug carriers in vascular stents and many other soft tissue applications. [238] [239] The most typical approaches of designing drug releasing surfaces are presented in Figure 5.25.

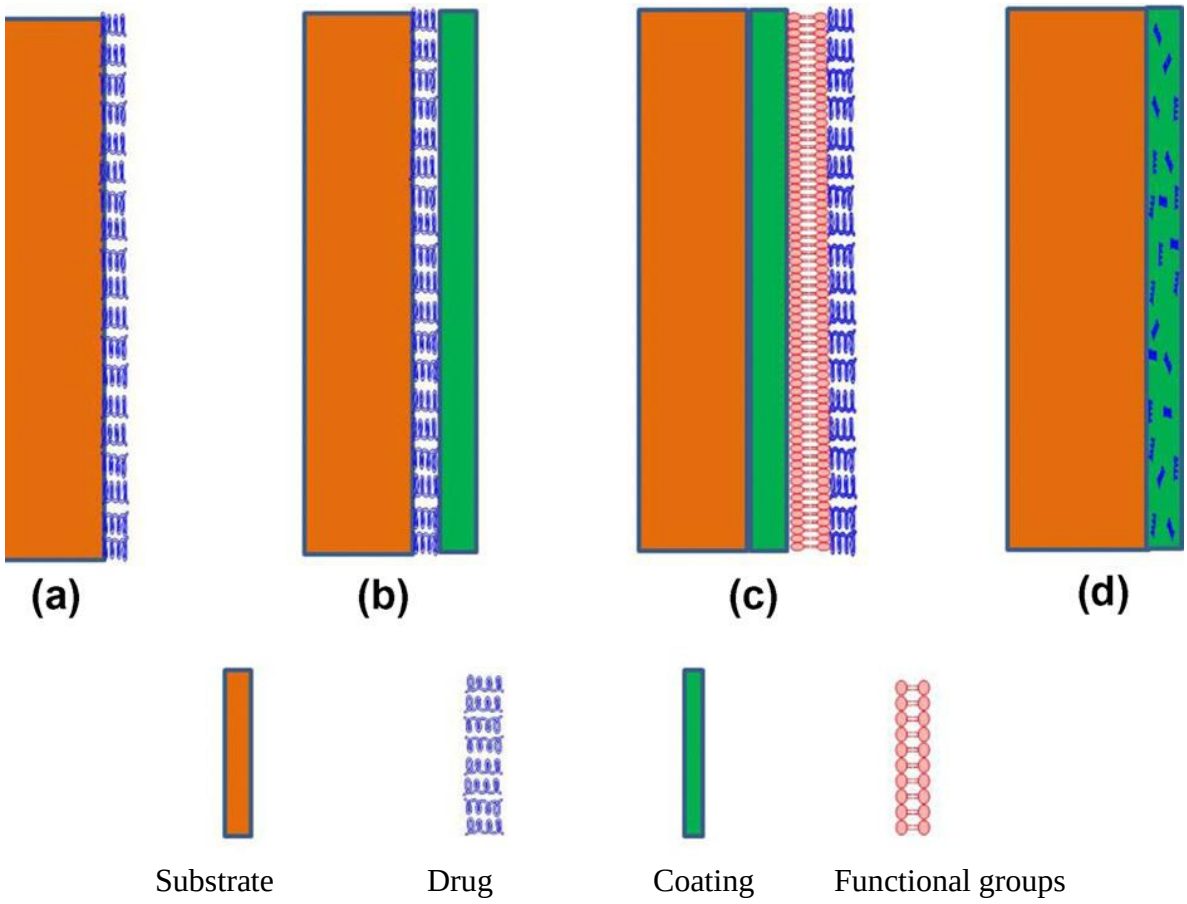


Figure 5.25. *Schematic illustration of different strategies to realise surface with drug release capability. (a) Drug molecules are directly coupled to underlying substrate; (b) Drug is interlocked between the substrate and coating layers in order to control the release of a drug; (c) Coating is deposited onto substrate and further modified with a functional, e.g. protein layer, which provides an appropriate bonding with a drug; (d) Drug is embedded to a coating matrix. Modified from [238].*

Structures produced by thermal spray technology have been under investigations for evaluating their viability of delivering antibiotic drugs. Unfortunately, no experimental evidence has been found with an aspect on cold spraying. The majority of these studies applied the strategy (c) in Figure 5.25., depositing the coating onto substrate and further modifying it with a functional layer of proteins which provides an appropriate bonding for the actual drug. An investigation by Taha et al. [240] aimed at fabricating plasma sprayed HA-coated Ti implant with a surface covered by cyclodextrins, which is a common carrier of therapeutic molecules. This drug was loaded with gentamicin, which was released in sustained manner offering a concentration adequate to eliminate the infection. The same

antibiotic was employed in a study conducted by Li et al. [241] whereby surface of a plasma sprayed Ti coating was treated with a solution containing type I collagen, which was thereupon treated with collagen fibres loaded with gentamicin. The bonding between collagen fibres and Ti coating was acquired by exposing the Ti coating to acidic environment, which resulted in formation of Ti-OH groups and hence, altered surface structure. As an outcome, a sustained release of antibiotics for over 30 days, and protection against *Staphylococcus aureus* was successfully acquired. Identical testing procedure was followed, likewise by Li et al. [242] to estimate the antibacterial capability of the gentamicin loaded collagen on plasma sprayed $CaSiO_3$. A Si-OH-rich structure formed at the top of the $CaSiO_3$ in the aqueous environment was the key to successful coupling of collagen fibres to $CaSiO_3$. The duration of release suggested that the amount of released antibiotics underwent an almost-linear increase until the third day and levelled off within 9 days in phosphate buffered saline. The release profile was consistent with the finding that gentamicin enclosed in the pores was released in the first place whereas gentamicin ionically bonded to carboxyl groups of the collagen showed more prolonged release. However, it has been indicated, that a post-operational infection typically arises within two weeks and therefore, inadequate delivery was provided. [242]

The most of the tissue that constitute the human body are composed of relatively soft tissues. From this perspective it is not surprising that major biomaterial applications such as cardiovascular stents make use of polymeric coatings [239]. Regardless of the predominance of degradable polymer coatings in the second generation drug delivery systems, very little has been published regarding thermal spraying of such polymers. Nonetheless, a full account of the viability of flame spray to degradable polymers was given by Chebbi [116]. A review made by Tan et al. [243] addresses hypersensitivity to free fibres released from degradable polymers as the major disadvantage that has remained unsolved but also identifies the promotion of desired cell specialisation as the future challenge for the developers of the endoprostheses.

5.4.8. Strategies of producing protein-modified surfaces

A wide range of different biomolecules are involved in a system beyond the cellular response to biomaterial. Therefore, in order to compensate the naturally deficient biocompatibility, mimicking the biological surfaces in molecular level has recently become an approach well-adopted to experimental studies. The biomimetic surface is often realised by attaching biological adhesive protein molecules on the surface. A representative example on such protein is collagen as discussed earlier but some other ECM-derived proteins such as RGD, fibronectin and chondroitin sulphate have been experimented [244] [245]. Mostly positive osteogenic effects induced by protein modified implants were reported after the implantation comparing to uncoated ones. Another way to improve the cellular response is to employ

adherent growth factors which encourage the given cell type to proliferate at its proximity. Included are proteins such as mitogenic IGF-1, FGF-2, PDGF-BB, collagen synthesis stimulator IGF-1, and osteoinductive BMP [246]. Viable immobilisation strategies for proteins are shown in Figure 5.26. as described by Bauer et al. [81] encompassing physical adsorption via electrostatic and Van der Waals interactions, entrapment, and covalent bonding.

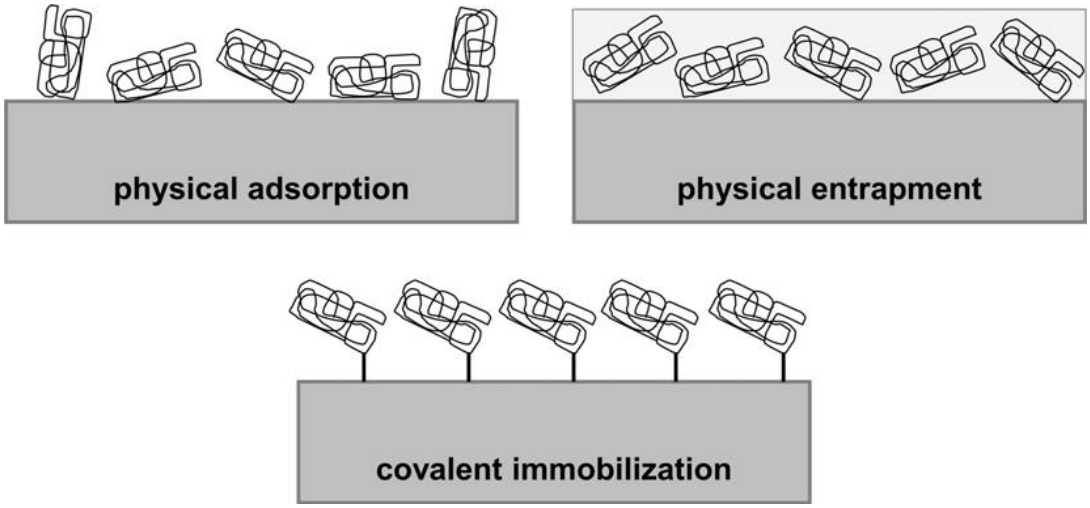


Figure 5.26. *Immobilisation strategies for biological molecules such as growth factors and enzymes [81].*

The surface of a biomedical CoCr alloy was modified with covalently bonded bone morphogenic protein BMP-7 by Tan et al. [247]. A proper attachment between the two was attained in passivating the CoCr surface and recruiting polydopamine proteins to establish an interlayer to attach the BMP-7 molecules to. A clear drop was recorded in the number of fibroblast adhesions on the BMP-7-containing surfaces. Similar procedure was carried out by Poh et al. [248] with BMP-2, which remarkably enhanced the levels of osteoblast differentiation marker and dramatically improved the deposition of Ca over three-week experiment.

Research papers dealing specifically with growth factors applied on sprayed coatings are few. However, some other drug-like molecules have been demonstrated on coating surfaces as exemplified by an *in vivo* study conducted by Peter et al. [249] bisphosphate known as zoledronate performed positive correlation to bone density in peri-implant area. These proteins were coupled to plasma-sprayed HA coating during immersion of the implant into a drug-containing solution.

5.5. Supplementary aspects

In addition to aspects discussed earlier, a few more criteria needs to be appreciated when medical biomaterials are addressed. Firstly, in order to fabricate surfaces with ideal structure and multifunctional properties, the requirements of mass production such as, short processing time, simple processing route, and reasonable cost should still be satisfied. With this respect, cold spray must be considered as a competitive technology. Secondly, the sterility of the biomedical surface must be verified, which might be realised by using techniques such as autoclaving, dry heat, radiation, or ethylene oxide. [81] Therefore, the biomaterial must not be destroyed if exposed under such treatment. Thirdly, the conflicts with highly sophisticated imaging systems need to be resolved, and in particular, the increasing diagnostic value of magnetic resonance imaging is widely acknowledged. From the diagnostic point of view a conclusion must be drawn that the biomaterials with high level of magnetic disturbance will be considered increasingly unattractive, since the MRI will be considered as a first line investigation for musculoskeletal implants. Hence, ferromagnetic metallic materials such as Ti alloys, Co alloys, and stainless steel, which cast large distortions to magnetic field and consequently, to MRI image, are not the primary alternative although their contrast is superior to many other materials in a computed tomography image. [44] [49]

6. IN-VITRO TESTING OF BIOCOMPATIBILITY

The concept of in vitro testing is a widely adapted term for biomedical testing that is performed in laboratory environment using biomimetic testing conditions. However, it is also important to appreciate the difference between in vivo and in vitro testing since no living organisms are involved in testing in vitro whereas animal models are essentially entailed as in vivo testing is employed. In this chapter, included are the essential in vitro methods which, with respect to thermal and cold spray coatings, may have relevance in biomedical engineering.

6.1. Tests conducted under Simulated Body Fluid (SBF)

Very often the testing of biomaterials is conducted under simulated body fluid, which consists of equivalent ion concentrations to physiological plasma as described in Table 5.1. Many types of simulated body fluids are utilised among which are the well-known Ringer's solution and Hank's solution with a slight variation in their balancing ion concentrations. Simulated body fluid has been well-adopted by experimental studies, especially those dealing with osseoinduction. Therefore, SBF solution defined by Kokubo et al. [250] has become the primary recipe in current experiments. The idea is to show whether the Ca^{2+} and $(PO_4)^{3-}$ ions nucleate on substrate as captured in Figure 6.1. The accumulation of a newly formed layer of apatite is regarded as a manifestation of the bone-induction capacity of a hard-tissue implant surface. Another indication of the chemical state of the biomaterial under immersion is the changes in ion concentrations, which are closely followed over the course of immersion. Ca^{2+} and $(PO_4)^{3-}$ ion consumptions are considered as viable indicators of bone forming activity. As a whole, this procedure often forms the basis for osseoinduction studies.

A high number of simulated body fluid –immersion experiments have been reported on plasma sprayed HA and bioactive glass coatings. However, due to a fact that majority of the bioactive materials are ceramic-based, cold sprayed coatings have been rarely involved. The principle of the SBF immersion -method is to demonstrate the mineralisation process in artificial physiological environment in observing the apatite nucleation upon the specimen surface over a period of 1, 7, 14 and 30 days [77] [93] [96]. Whether the mineralisation occurs on the test samples is typically revealed by scanning electron microscopy which is often combined with energy-dispersive spectrometry studies providing information about the Ca/P ratio that is supposed to correspond to 1.67, which is the compositional ratio for crystalline apatite found in natural bone according to ISO 13779-3 standard. Another popular method

capable of detecting the phases dissolved during the soaking is x-ray diffraction measurements which, in addition to SEM and EDS, must be considered as a standard method for characterisation of biomedical hard-tissue coatings. [91] [93] [96] [250] A third strategy of detecting mineralisation in simulated body fluid is via an ion concentration study, which might be carried out using inductively-coupled plasma atomic emission spectroscopy as demonstrated by Cannillo et al. [96]

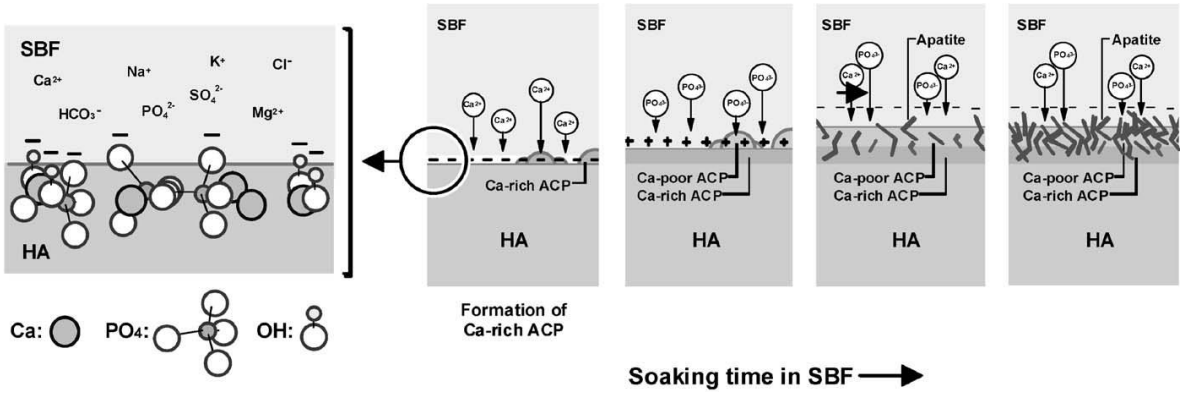


***Figure 6.1**. The formation of the bone-like apatite layer on the HA coating in SBF [251].*

Various alterations in critical coating properties such as fatique and adhesion strength, and corrosion and wear resistances are linked to environmental factors. Therefore, mechanical testing performed after SBF immersion is often an important part of the evaluation of the medical coatings. It is an effective way of exposing the structural defects or coating impurities, which has commonly been identified as the underlying cause for a dramatic drop of the adhesion strength with thermal sprayed coatings following an immersion into SBF. Such effect is closely related to phase transformations that take place in high temperatures accounting for a drop to as low as third of the original values for adhesion strength as reported by Melero et al. [77] with HVOF-sprayed HA-TiO_2. In terms of wear testing, SBF is often engaged in pin-on-disc or impact sliding tests as realised by Cheng et al. [252] because considerable decrease in friction coefficient is observed in the presence of a fluid. Moreover, SBF has been an ideal in vitro environment for corrosion studies carried out in open circuit potential measurements by Al-Mangour et al. [40], and Zhou et al. [39]. Also in determining the degradation rate of the Mg alloys SBF studies combined with hydrogen evolution measurements, electrochemical measurements, and microtomography have provided valuable data [187].

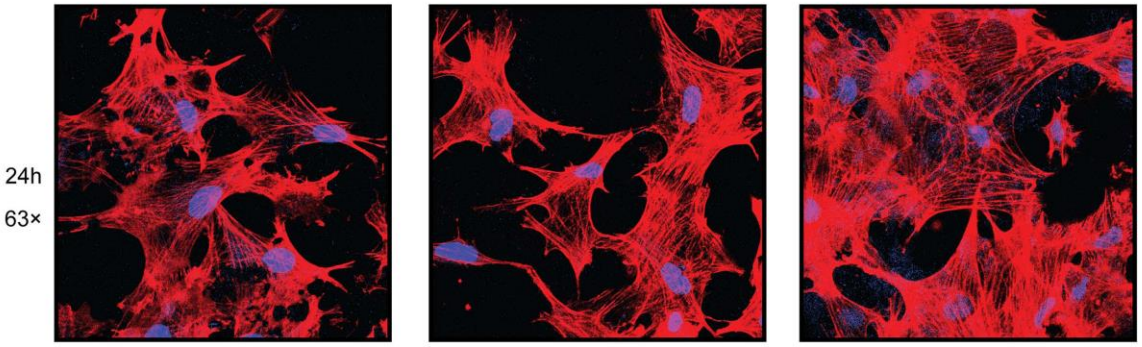
6.2. Research methods for cell culture

At present, biomedical materials research mostly relies on detecting the changes in cultivated cells in response to foreign object. Each experiment is carried out using selected population of cells. Deciding which cell line will be applied depends on the target application which in context of hard-tissue implants account for the wide use of mesenchymal stem cell -derived cell lines. Cell quantity, morphology, adhesion, and size are valid indicators for the type of the contact, and help predicting the long-term viability. However, the effect of the contact is expressed by the changes in cell metabolism, which provides more specific clues for investigating the cell-biomaterial relationships. The basic research methods used in this area will be introduced here.

6.2.1. Cell dimensions by microscopic techniques

Fluorescent microscopy is one of the most popular methods to gather information about the living cells. The principal idea is to attach an autofluorescent molecule to biomolecule of a cell in order to gain contrast under external UV light. Therefore, the targeted biomolecules will view enhanced as the fluorescent markers are coupled to them. [118] Although the number of fluorescent markers is limited, the technique is essential in acquiring information on detailed cellular components such as organisation of the cytoskeleton. Hence, with respect to biomaterials the main interests of fluorescent microscopy study are cell adhesion, morphology, quantity, and size. In context of thermal spray coatings it has been utilised for osteoblast morphology studies by Lee et al. [20], Melero et al. [77] Shtansky et al. [214] and Tang et al. [68] which is visualised in Figure 6.2. revealing the arrangement of cytoskeletal actin filaments of human bone marrow stromal cells. Actin filament findings supported for more flattened and tensed cell structure on Ta coating (c) in comparison with the cells grown on Ti (a), (b).

In a similar way, optical microscopy and electron microscopy have their own contrast agents that might be specific for a certain type of target proteins. [118] Such colorimetric examinations are frequently carried out: Liu et al. [92] investigated the protein adsorption of the cell adhesion –protein fibronectin onto cold-sprayed coating by using a stain which selectively binds to fibronectin enhancing the transmission electron microscope images.



a) b) c)

***Figure 6.2**. Confocal laser scanning microscope image on the morphology of the fluorescent phallotoxin –stained cytoskeletal actin filaments on (a) pure Ti, (b) vacuum plasma sprayed Ti coating, and (c) vacuum plasma sprayed Ta coating [68].*

Atomic force microscopy (AFM) is a potential method for imaging biological tissue because it is free of the requirement for ultra-high vacuum, unlike the other candidates for high resolution imaging such as scanning electron microscope (SEM) or transmission electron microscope (TEM) [118]. It is a beneficial technique in surface profiling of intricate structures such as the metal surface covered with immobilised bone morphogenic proteins (BMP-II) in a study of Poh et al. [248] Advantageously, force of surface adhesion might be obtained by AFM technique, but as a backside very limited variation in surface depth is allowed. [253]

6.2.2. Protein expression studies by enzymatic methods

An extremely high number of different proteins are produced by a living cell encompassing a subcategory of enzymes that catalyse biological reactions. The activity of these enzymes abundant in intracellular fluid, is greatly dependent on the state of the cell functions. Therefore, enzymatic diagnostics has become one of the primary approaches to study cell response. A typical test is based on reaction catalysed by an enzyme that is readily present in a cell and introducing an external substrate to the cell turns it into a product. The concluding analysis is done by measuring the concentration of the final product, which is often realised by means of spectrophotometry. The principle of this measurement is to detect the change in photon absorbance using wavelength specifically absorbed by the product molecules. [127]

Methyl thiazole tetrazolium (MTT) is a quantitative test used as proliferation and cytotoxicity indicator in estimating cell-biomaterial viability. [254] [255] Also other tetrazolium compounds exist such as XTT, MTS, and WST-I, but are not as established as MTT assays for testing cell-biomaterial biocompatibility. In contact with a living eukaryotic cell tetrazolium undergoes a transformation to formazan, which is a coloured compound with an

absorbance maximum of 570 nm. Therefore, as MTT assay solution is introduced to living cells, tetrazolium penetrates to the cell and is turned to formazan by some unknown mitochondrial enzymatic mechanism. Such reaction is only possible in active living cells. As an outcome, the expressed cell activity is interpreted as an indication of cell proliferation, because there is a direct correlation between the cell number and the measured level of absorbance. [254] Naturally, the extent of the reaction depends on the length of the incubation period and for this reason, the absorbance measurements are repeated in time intervals of 1, 3, and 5 days as adopted by Lee et al. [20] and Liu et al. [92] with cold sprayed HA coatings using human bone mesenchymal stem cells and human osteoblast cells respectively. Moreover, the MTT assay test was incorporated to investigations on Ta coatings fabricated by plasma spraying by Yu et al. [100] and anodisation by Wang et al. [69].

Osteogenic differentiation is one of the most important indicators of evaluating the performance of a hard tissue implant. In order to confirm the level of differentiation alkaline phosphatase activity (ALP) is measured. Alkaline phosphatase is an intracellular enzyme responsible for removing the phosphate groups and is associated with bone forming activity of the osteoblasts. Therefore, it is considered as an indicator of osteogenic differentiation when extensive ALP activity is detected. In practice, induced cell lysis and enzyme release into alkaline solution is the initial step of the testing procedure. Thereafter, paranitrophenyl phosphate (PNPP) substrate is introduced into the solution. The PNPP substrate reacts catalysing alkaline phosphatase yielding p-nitrophenol which as a colourful end-product is easily quantified by spectrophotometric reader device. [20] [256] Such testing procedure has been described by authors including Liu et al. [92] with human osteoblasts on cold sprayed HA/graphene coatings and Lee et al. [20] with human bone mesenchymal stem cells cultivated on cold sprayed HA coatings which is presented in Figure 6.3. An identical in vitro test scheme was undertaken for plasma sprayed Ca-Si-Zn coating by Yu et al. [100] with osteoblast-line cells. Dissimilar to these studies, ALP activity was determined using ALP staining and antibody-assisted immunofluorescence techniques to identify human bone marrow stromal cells on vacuum plasma sprayed Ta by Tang et al. [68].

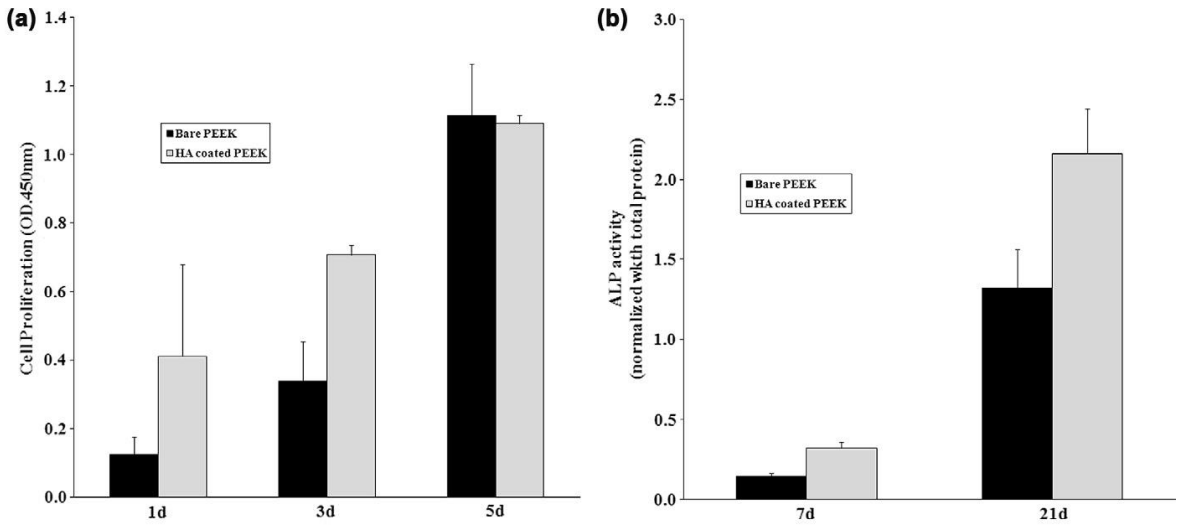


Figure 6.3*. Human mesenchymal stem cells cultivated on bare PEEK substrate versus PEEK substrate coated with HA by cold spray technology. a) Cell proliferation based on photoabsorbance of the MTT assay end-product formazan. b) Activity of the differentiated cells according to photoabsorbance of the ALP assay end-product p-nitrophenol [20].*

6.2.3. Gene expression studies by reverse transcription polymer chain reaction

Polymer chain reaction is a technique that allows a rapid and accurate multiplication of a known sequence of DNA. It is widely used in investigating whether the given genes are active or suppressed. In the biological process of transcription, the active genes in DNA are encoded into mRNA and by detecting these mRNA strands the activity of the corresponding gene can be ascertained. In the biological cell, these mRNA strands may further be translated into proteins and channelled through post-translational modification prior to achieving the definitive functional structure. However, by means of reverse transcription polymer chain reaction (RT-PCR) –technology it is possible to convert the mRNA into complementary DNA (cDNA) and multiply these strands for millions of times. Through polymer chain reaction it is possible to produce extremely high number of cDNA strands, which is necessary in order to complete the following step: The strands are analysed with gel methods dedicated to discriminate and identify the strands. This is an excellent in vitro technique for examination of gene expression that is slightly different to each cell type. For this reason, gene-specific RT-PCR method is an ideal tool to appreciate the level of differentiation. As a disadvantage, nothing conclusive concerning the protein expression can be deduced based on RT-PCR studies since the correlation between mRNA and final protein is highly dependent on post-translational modifications. [118] RT-PCR scans were run after 1, 2, and 3 weeks cultivation by Yu et al. [100] as an attempt to study the evolution of gene expression of osteoprogenitor cells on plasma sprayed $Ca_2ZnSi_2O_7$. As a result differentiation-inducive

effect was concluded as evidenced by upgraded levels of mRNA of osteoblast-specific genes: alkaline phosphatase, procollagen α1(I), and osteocalcin, as well as upgraded levels of mRNA of growth factors such as IGF-I and TGF-I which are abundant in osteoblasts. The respective diagrams are represented in Figure 6.4. Furthermore, another study was conducted by Xie et al. [220] wherein the RT-PCR method was employed in verifying the differentiation of human marrow stem cells on anodised plasma sprayed titanium coating. Osteocalcin, osteopontin, alkaline phosphatase, and type I collagen operated as indicative markers. In a similar fashion, quantification was implemented by Lee et al. [20] for osteoblast differentiation markers such as alkaline phosphatase, bone sialoprotein, and runt-related transcription factor 2 (RUNX2). The results suggested prioritised osteoblast differentiation on hydroxyapatite coating according to RUNX2 and sialoprotein expression.

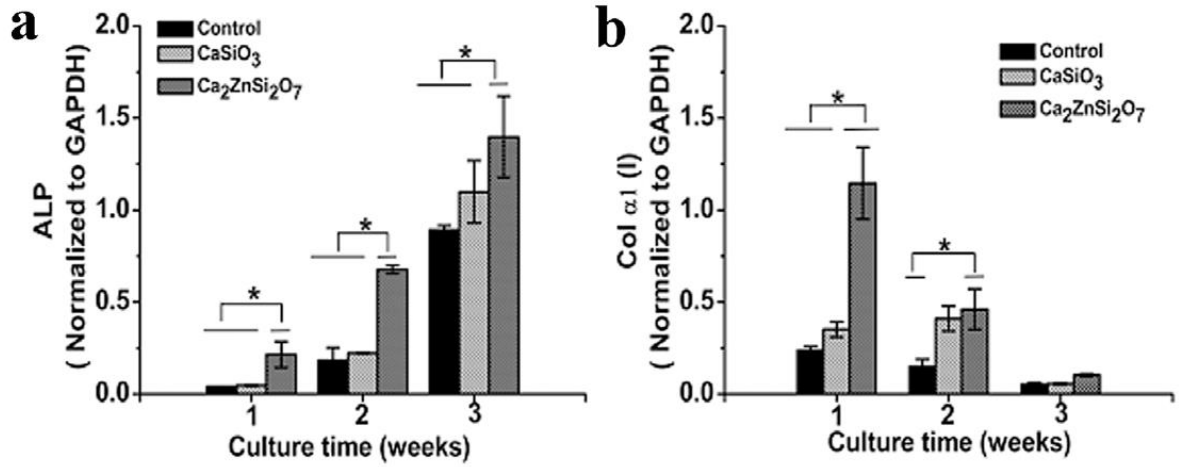


Figure 6.4. *mRNA expression levels of a) alkaline phosphatase and b) procollagen α1(I) obtained by RT-PCR from osteoprogenitor cells grown on plasma sprayed $Ca_2ZnSi_2O_7$ [100].*

6.3. In vitro tests for exposing antimicrobial activity

There are at least three alternative methods for bacterial viability analysis: Colony-forming units –counting, metabolic activity assays, and molecular probe assays. Counting of colony-forming units (CFUs) is a conventional method that is identical to optical microscopy method introduced in chapter 6.2.1. for eukaryotic cells. The idea is to cultivate bacterial cells over a certain period on a test object followed by staining of the bacteria with a selective stain. Thereafter, the final conclusion for antibacterial activity is made on the basis of counting the area overlaid by the bacterial cells in comparison with the control specimen. It is sensitive to minute counts of bacteria, but as a drawback may lead to an underestimation of colony-forming units due to aggregation and superposition of the bacteria. [257] Counting of bacterial colonies was performed by Li et al. [258] for *Staphylococceus aureus* cultivated on a plasma sprayed $Ca_2ZnSi_2O_7$. The results shown in Figure 6.5. suggested that the coating clearly displayed an effect against *S.aureus*.

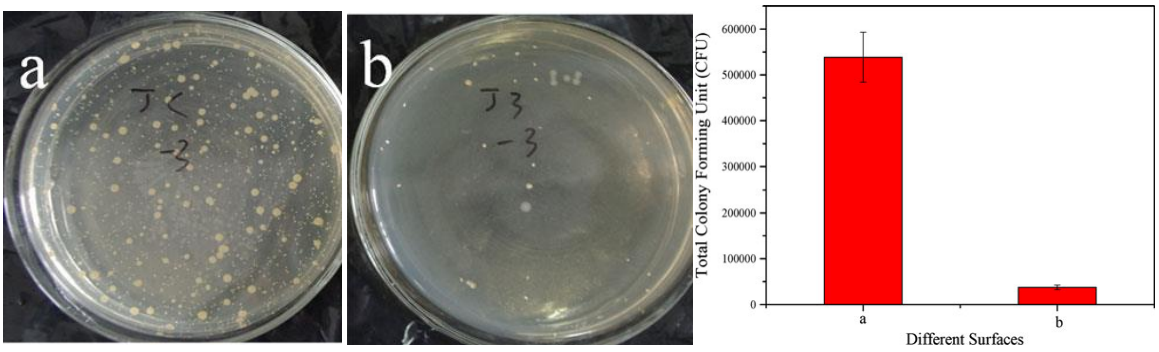


Figure 6.5. *Bacteria colonised on a) pure Ti-6Al-4V and b) plasma sprayed $Ca_2ZnSi_2O_7$ coating. The colony-forming unit counts are presented as a schematic diagram. [258]*

Loyal to CPU counting protocol, antibacterial capacity of plasma sprayed TiO_2- and $CaSiO_3$-based coatings have been under evaluation by Li et al. [231] [233] whereas Zn-modified coatings produced by cold and plasma spray technology were investigated by Tamai et al. [228] and Li et al. [230]. A wide range of experimental studies associated with cold spraying of antibacterial coatings has been published by Sanpo et al. [227] [224] [226] [225] that were elaborated in chapter 5.4.5. In these studies, an adaptation of CPU counting method served as a primary test to quantify the survived *E. Coli* bacteria.

Molecular probe assay is an alternative method of quantifying antibacterial efficacy. Such assay relies on fluorescent molecules that selectively couple to receptor molecule of a given type as was outlined in chapter 6.2.1. This method was applied by Roy et al. [259] using Live/Dead BacLight Bacterial Viability Kit to discriminate the dead and the living cells placed on a plasma sprayed 2%-Ag-containing HA coating. Two different molecules were

introduced to bacteria: green fluorescent nucleic acid stain SYTO9 to expose the living cells and red fluorescent light –emitting propidium iodide component selective to dead cells. Under a confocal laser scanning microscope, a categorisation according to whether the adherent bacteria were alive or dead was done. The resulting images are shown in Figure 6.6. The same protocol was followed by Melero et al. [77]

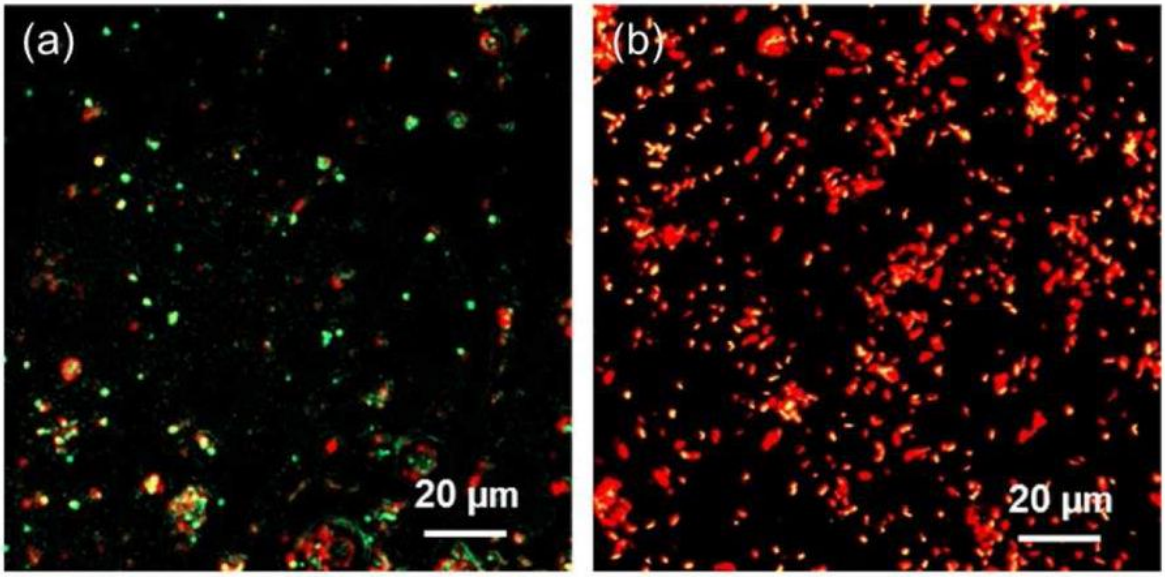


Figure 6.6. *Molecular probe assay images of bacteria colonised on a) pure HA coating with predominantly greenish colour of the colonies, and b) 2-wt% Ag-doped HA coating with a considerable quantity of dead (red) bacteria [259].*

In theory a third strategy of assessing the antibacterial effect is via metabolic activity assays, which are dedicated to characterise the change in activity of the bacterial cells with respect to antibacterial surface. [260] [261] However, for some reason bacterial activity tests are rarely carried out using sophisticated metabolic activity assays and instead, the role of simple CFU method in overall in vitro evaluation of antibacterial surfaces seems to be pronounced as it is used routinely.

6.4. In vitro testing of photocatalytic capacity

Heterogeneous selection of both non-bacterial and bacterial methods exist for testing photocatalytic activity. Bacterial methods are essentially identical to those listed in chapter 6.3. However, bacterial culture is not necessarily needed to determine the rate of photocatalysis for it might be measured through various methods including the change in pH when a photocatalytically active object is immersed in a solution rich in dissolved indicator. The change in pH results from the acidic end-product of photocatalytically induced reaction. Such an approach was decribed by Burlacov et al. [167] who generated a photocatalytic reaction on an immersed TiO_2 coating by irradiating the surface with a photoreactor. The 4-chlorophenol (NaClO4) –indicator underwent a chemical reaction releasing acidic HCl which resulted in the change in pH.

Photocatalytic conversion of methylene blue under UV irradiation was the method of choice to examine photocatalysis on suspension plasma sprayed TiO_2 coating by Bannier et al. [73]. The degradation of methylene blue due to photocatalysis was followed by equivalent decline in absorbance of the solution, which is easily determined by spectrophotometer. The results are shown in Figure 6.7. The same principle was exploited by Yang et al. [236] with cold sprayed TiO_2. The decline in concentration of photocatalytic agent acetaldehyde under UV radiation was recorded in ten-minute intervals by gas chromatography.

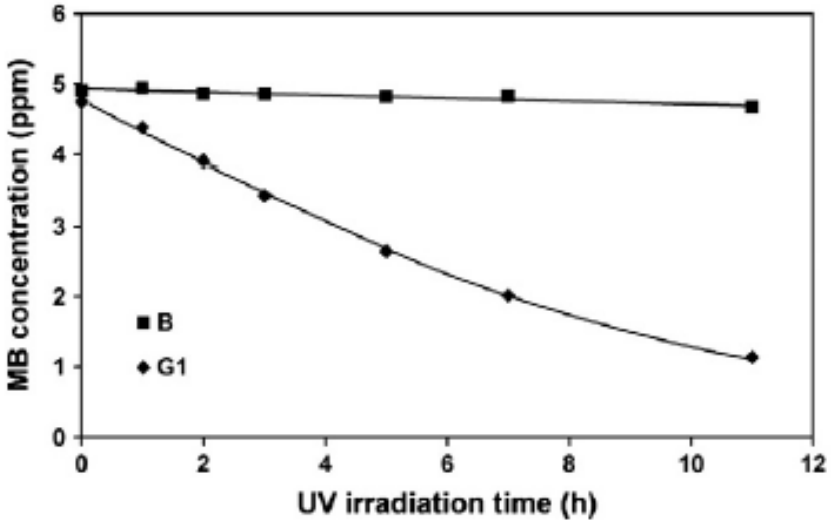


Figure 6.7. *Photocatalytic efficiency in terms of decreasing concentration of methylene blue solution containing G1) suspension plasma sprayed TiO_2, and B) uncoated reference [73].*

In a similar way Ivanova et al. [237] studied photocatalysis of a cold sprayed TiO_2 under UV(A) radiation by means of degradation of organic acids: oxalic and dichloroacetic acids. Two independent techniques was exploited: Static pH technique was used to monitor changes in catalytic degradation-product i.e. hydroxide-ion concentration, and high performance ionic chromatography provided information on concentrations of essential anions such as reactants. An illustration of a photocatalytic degradation behaviour is seen in Figure 6.8. as realised by Ivanova et al. [237]

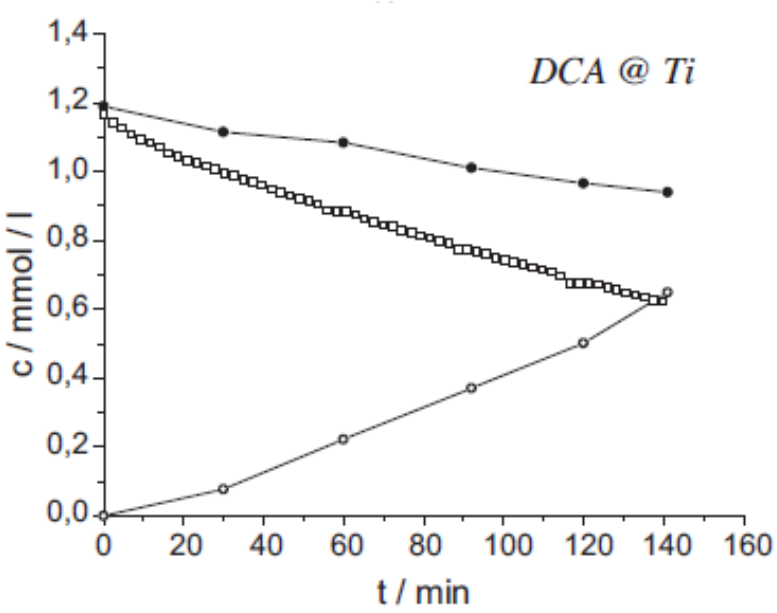


Figure 6.8*. Changes in concentrations during photocatalytic degradation on cold sprayed TiO_2 coating. The two uppermost curves demonstrate the declining dichloroacetic acid concentration measured by (•) high performance ionic chromatography and (□) pH static method. The ascending concentration curve at bottom was recorded for released (◌) chloride by high performance ionic chromatography [237].*

6.5. Summary of in vitro research methods

Some routine in vitro methods have been introduced in chapter 6. Bioactivity and more precisely osseoinduction is widely demonstrated by soaking the specimen in simulated body fluid. It is a straightforward test and hugely important in pointing out active bone formation on orthopaedic coatings. Secondly, cell viability studies were discussed. Basically, these in vitro methods are perfomed for cultivated cells and might be split in two groups: microscopy methods and expression studies. The first group, microscopy, is excellent tool in studying cell size, quantity, morphology, and adhesion whereas gene and protein expression studies

supply information about cell metabolism and hence, are fantastic experiments in assessing activity and differentiation of a cell. Together, these experiments are the backbone of biocompatibility. Third category: antibacterial properties of biomedical coatings are regularly being examined with conventional CFU –counting and, although other applicable methods exist via which the metabolic state of a bacterium might be figured out, vast majority of the research has merely concentrated on finding the colonies. The fourth category: photocatalysis has been included regardless of the fact that it may not play a role in intracorporeal applications. Apart from this, it is under active research for many medical applications, it is suitable for cold spray technology, and displays favourable cell response. However, the variety of methods used for exploring photocatalytic efficiency likely reflects the short history of such coatings. The central points have been extracted in Table 6.1.

Table 6.1. *Comparative matrix of in vitro techniques for biomedical coatings.*

	Desired information	Methodology	Technique	Principle	Advantage	Disadvantage	Authors
Mineralisation	rate and amount of apatite precipitation	Solution ion concentration monitoring in SBF immersion	Spectroscopical methods	Phosphate and calcium ions nucleate leading to change in their dissolved concentrations	Gives an estimation for quantity of precipitation		Cannillo et al.[96]
		Precipitate qualification by phase characterisation after SBF immersion	Electron microscopy, Energy dispersive spectrometry (EDS), X-ray diffraction (XRD)	Ca/P ratio of natural apatite equals to 1.67	Sensitive to detect changes in apatite composition		Bolelli et al. [91][93] Cannillo et al.[96] Melero et al.[77]
Cell viability	Adhesion, morphology, quantity and size of the cells	Labelling of structural proteins	Fluorescence microscopy	Cells labelled with autofluorescent molecules become visible in microscope	Use of different colours simultaneously, tracing of genetically inherited markers is possible	Only the labelled section of a cell becomes visible	Lee et al.[20] Melero et al.[77] Tang et al.[68]
		Targeted staining of structural molecules	Optical microscopy, Electron microscopy	Cells labelled with specific contrast agent are seen enhanced in microscope	Versatile because high number of contrast agents have been developed	Information based only in two-dimensional view, ultra-high vacuum of TEM is problematic with cells	Liu et al.[92] Melero et al.[77]
		Imaging of biological surface molecules of adhered cells, measuring adhesion force	Atomic force microscopy	Fine details of cell-material binding site in 3-D microscope image	Extremely high resolution,	Ideal only for very smooth surfaces	Poh et al.[248]
	Proliferation, cytotoxicity	Measuring the level of enzyme production in a cell	MTT assay	Tetrazolium is converted to formazan in a reaction catalysed by the enzyme that is being quantified	Specific information of cell functioning, quantitative test	Assay conditions may alter metabolic activity, enzymatic mechanism partly unknown	Lee et al.[20] Liu et al.[92] Wang et al.[69] Yu et al.[100]
	Osteogenic differentiation	Measuring the level of ALP enzyme production in a cell	ALP assay	PNPP is converted to p-nitrophenol in a reaction catalysed by intracellular alkaline phosphatase	Specific quantitative information on cell characteristics	Conclusion drawn on the basis of expression of a single enzyme	Lee et al.[20] Liu et al.[92] Tang et al.[68] Yu et al.[100]
	Differentiation	Expression of selection of genes	RT-PCR	mRNA is encoded into cDNA, and further multiplied unlimitedly	Superior method for thorough investigation of differentiation when using multiple DNA sequences	Due to post-translational modifications gene expression does not always equal to protein expression	Lee et al.[20] Xie et al.[220] Yu et al.[100]
Antibacterial capacity	Rate and extent of bacterial death	Staining and microscopy	Colony-forming units counting	Spread of the stained bacterial colonies are visible under microscope	Simple, accurate in detecting fine bacterial colonies	No information on metabolic state of a cell but only whether the cells exist, cells detected only in planar view	K. Li et al.[223][230] B. Li et al.[233][242] Tamai et al.[228] Sanpo et al.[224-227]
	Extent of induced bacterial dysfunctions	Fluorescent staining and microscopy	Molecular probe assay	Cellular molecules labelled with fluorescent component become visible under microscope in dark	Quantitative estimation on both dead and living cells	Photobleaching	Melero et al.[77] Roy et al.[259]
		Measuring concentration of a substance characteristic for metabolically active bacterium	Metabolic activity assay	Expression of the proteins in bacterium depends on environmental factors	Provides information of actual bacterial activity, Overlapping colonies are taken into account		
Photocatalytic activity	Rate of photocatalytic reaction	Solution pH monitoring	pH electrode	Conversion of an organic substrate into acidic/basic end-product	Simple test setup		Ivanova et al.[237] Burlacov et al.[167]
		Solution absorbance monitoring	Spectrophotometry	Conversion of an organic substrate into optically divergent end-product	Simple test setup		Bannier et al.[73]
		Solution ion-concentration monitoring	Chromatography	Conversion of an organic substrate into ionic end-product	Monitor of a wide range of molecules	More complex test setup	Yang et al.[236] Ivanova et al.[237]

7. CONCLUSION

Generally speaking, there is an ongoing trend towards the integration of biofunctionalities and materials that extends over the field of biomedical cold spraying. Hence, during the short history of cold spray technology the coating performance testing has increasingly concentrated on developing surface properties that encourage normal cell growth not compromising with coating durability and safety. According to experimental demonstrations presented in this study cold spray method is a competitive technology that holds potential in producing biomedical coatings. The central findings are summed up in Table 7.1.

1. The importance of inter-particle bonding formation was manifested by a loss of tensile and fatigue strength subsequent to cold spray deposition. Fatigue endurance of the biomedical cold sprayed coatings was unexpectedly comparable to the values of plasma sprayed coatings, which are known to suffer from combined effects of wear and fatigue such as delamination. However, a heat treatment markedly improved the fatigue endurance. These results were surprising, because of the conflict with the hypothesis based on the earlier findings of compressive residual stress of the coating structure. Positively, no dramatic differences was found concerning mechanical properties, namely adhesion strength, fatigue endurance, and elastic modulus between the cold spray and thermal spray coatings.

2. Potential of the cold spray method to create well-adhered biomedical coatings was shown with mixed Ti and HA particles. In contrast, the adhesion strength remained low when ceramic TiO_2 and HA particles were accumulated on polymeric and polymer-composite substrates. However, clinical problem of delamination related to thermal spray coatings was underpinned by the decrease in adhesion strength during soaking in SBF. To establish an intimate bonding no dissolvable phase of HA is formed with cold spray.

3. Fabrication of nano-structured coating was repeatedly proposed as a realistic approach to enhance material properties in biomedical setting. Nano-sized feedstock resulted in improvements in overall structural homogeneity manifested by wear resistance and fatigue strength. Even more radical is the impact of nano-structures on cell functions since it was remarkably more favourable on nano-crystalline features compared to micron-size structures. The main attributes for this effect were topographical dimensions and larger surface area. Furthermore, an intense antibacterial effect was linked to nano-structures.

Table 7.1. *Property-based listing of the important findings of biomedical thermal spray coatings.*

	Author	Coating method	Coating material	Substrate material	Central finding	Additional remarks
Tensile strength	Vo et al. [29]	cold spray	Ti-6Al-4V	Ti-6Al-4V	Low tensile strength in cold sprayed condition, Annealing treatment considerably enhances tensile strength	
Fatigue strength	Laonapakul et al. [53][54]	plasma spray	HA	Ti	Susceptibility to delamination in SBF immersion	Clinically identified problem
	Gledhill et al. [84]		HA	Ti		
	Cizek et al. [27]	cold spray	Ti	Ti-6Al-4V	Reduced fatigue life due to tensile residual stress and increased surface roughness, Annealing treatment enhanced fatigue strength	Fatigue strength reduction might be compensated by using nano-sized powders
	Price et al. [50]		Ti	Ti-6Al-4V		
	Al-Mangour et al. [38]		stainless steel 316L			
Adhesion strength	Melero et al. [77]	HVOF spray	TiO_2+HA	Ti-6Al-4V	A drop in adhesion strength as a consequence of SBF immersion	
	Sun et al. [165]	cold spray	Ti	Titanium	Good adhesion strength was displayed by highly porous coatings	Porosity level has relevance in bone ingrowth
	Qiu et al. [164]		Ti/HA	Titanium		
	Binder et al. [166]	cold spray	Ti	Al	An adequate bonding for biomedical applications is easily acquired with titanium particles	
	Price et al. [50]		Ti	Ti-6Al-4V		
	Choudhuri et al. [162]		Ti/HA	Al		
Corrosion resistance	Trentin et al. [26]	cold spray	Ti	CoCr	Cold spray coating acted as a barrier demonstrating a protective effect	
	Al-Mangour et al. [40]		stainless steel 316L/CoCr	stainless steel 316L		
	Koivuluoto et al. [182]		Ta	pH electrode		
	Noorakma et al. [62]	cold spray	HA	Mg	Demonstration of degradation of deposited magnesium alloy in simulated body fluid	Cold spray coatings may potentiate controlled magnesium degradation
Wear resistance	Chen et al. [179]	cold spray	Al/CNT	Al	Involvement of carbon nanotubes remarkably reduced the rate of wear	
	Kang et al. [23]		Al/CNT	Al		
Porosity	Sun et al. [165]	cold spray	Ti	Ti	Favourable pore distribution was obtained using porogen-assisted techniques	
	Qiu et al. [164]		Ti/HA	Ti		
Surface topography	Liu et al. [92]	cold spray	HA/graphene	Ti	Nanocrystalline structure was preserved owing to low thermal input	Favourable cell response is closely related to nanostructured surfaces
	Bae et al. [210]		Cp-Ti	steel		
Wettability	Shtansky et al. [214]	cold spray	Ti	Ti	Hydrophobic behaviour was exhibited by coatings composed of coarse particles	Hydrophilic surface up-regulates adsorbed proteins and cell adhesions
Antibacterial capactiy	Sanpo et al. [224-227]	cold spray	HA-Ag/PEEK, ZnO-Al, ZnO/Ti		Coatings demonstrated antibacterial effect	Antibacterial particles interfere with bacterial cell membrane
	Tamai et al. [228]		ZnO-glass	Ti-6Al-4V		
	Vucko et al. [229]		Cu	HDPE		
Photocatalytic activity	Burlacov et al. [167]	cold spray	TiO_2	PS	Excellent rate of photocatalysis was demonstrated	Low thermal input helps preserving the preferred phase
	Ivanova et al. [237]		TiO_2	stainless steel, Cu, Ti		
	Yang et al. [236]	cold spray	TiO_2	stainless steel	Owing to high purity cold sprayed anatase exhibited superior catalysis to HVOF reference	
Anti-inflammatory agents	Taha et al. [240]	plasma spray	HA	Ti	Drug carriers were successfully immobilised onto coating	
	Li et al. [241][242]		Ti	Ti		

4. In terms of corrosion and degradation, dense coatings are easily fabricated by cold spray technology enabling corrosion avoidance through barrier. The deposition of temperature-sensitive Mg, which is under active research for biodegradable applications. Regardless of whether the magnesium is used as a coating or a substrate, cold spray technology would presumably be beneficial in creating structure with controlled porosity. Such implants with controlled degradation rate are constantly pursued in context of hard tissue implants.

5. Implant fixation has traditionally been acquired by means of bone ingrowth into a porous coating. Successful fabrication of highly porous cold sprayed coatings was demonstrated in two separate studies, which both documented convincing adhesion strength.

6. Very few systematic approaches have been taken to investigate the effect of topographical features on biocompatibility regarding cold sprayed coatings. An ideal surface topography is essential in encouraging protein adsorption and cell attachment. Cold spray might be the key to avoid grain growth and preserve the nano-structured topography. An approach of contrast is to develop surface with controlled topography and surface charge to avoid adhesion of any organic material, which further inhibits bacterial habitation and development of stenosis.

7. Wide range of Ag, Cu, and Zn-based antibacterial coatings were produced and validated on both metallic and polymer substrates.

8. Potential of cold spray method for deposition of photocatalytic TiO_2 coatings was evident: As a low thermal input –method cold spray no anatase-to-rutile transformation occurs and as a consequence, high rate of catalytic degradation was shown.

9. In the future, drug releasing ability will be the emphasised with the medical implants. Hence, the involvement of different drug carriers such as carbon nanotubes is necessary for future investigations and opens up new prospects. Also, the role of regenerative medicine i.e. stem cells in relation to biomedical coatings should be appreciated when future coatings are designed.

At present the biomedical hard-tissue coatings are predominantly manufactured by plasma spraying. From that perspective, the future challenge with cold spraying is to tailor coatings with optimised set of properties for specialised medical targets. Therefore, the future efforts with orthopaedic coatings should be aimed at

1. enhancing biocompatibility by using nano-size powders composing of material combinations such as TiO_2-HA, or graphene-HA. Additionally, Mg particles might assist in creating a porous surface.
2. improving the adhesion between e.g. HA or TiO_2-based thermal spray coatings and composite substrate displaying low elastic modulus.
3. developing wear-resistant articular surfaces from materials that show clinically acceptable long-term response.

REFERENCES

[1] K. H. Kim, M. Watanabe, K.-H. Kim and H. Katanoda, "Current Status and Future Prospects of Warm Spray Technology," *Journal of Thermal Spray Technology,* vol. 20, no. 4, pp. 653-676, 2011.

[2] J. Davies, Handbook of Thermal Spray Technology, United States: ASM International, 2004.

[3] "Dip, Barrier and Chemical Conversion Coatings: Thermal Spray Coatings: Thermal Spray Processes," in *ASM Metals Handbook*, 2002.

[4] P. Vuoristo, "Terminen ruiskutus – menetelmät, pinnoitteet ja teolliset sovellukset," *Hitsaustekniikka,* vol. 54, no. 5, pp. 5-18, 2004.

[5] M. L. Berndt and C. C. Berndt, "Thermal Spray Coatings," in *ASM Handbook 13A, Corrosion: Fundamentals, Testing and Protection,* ASM International, 2003, pp. 803-813.

[6] A. Papyrin, Cold Spray Technology, Elsevier Ltd., 2007.

[7] R. G. Maev and V. Leshchynsky, Introduction to Low Pressure Gas Dynamic Spray, Weinheim: WILEY-VCH Verlag GmbH & Co., 2008, pp. 1-4, 135-140.

[8] H. Singh, T. S. Sidhu and S. B. S. Kalsi, "Cold spray technology: future of coating processes," *Frattura ed Integrita Strutturale,* vol. 22, pp. 69-84, 2012.

[9] T. Hussain, "Cold Spraying of Titanium: A Review of Bonding Mechanisms, Microstructure and Properties," *Key Engineering Materials,* vol. 533, pp. 53-90, 2013.

[10] H.-J. Kim, C.-H. Lee and S.-Y. Hwang, "Fabrication of WC-Co coatings by cold spray deposition," *Surface and Coatings Technology,* vol. 191, no. 2-3, pp. 335-340, 2005.

[11] K. H. Kim, S. Kuroda, M. Watanabe, H. F. R. Huang and H. Katanoda, "Comparison of oxidation and microstructures of warm-sprayed and cold-sprayed

titanium coatings," in *Proceedings of the International Thermal Spray Conference*, Hamburg, 2011.

[12] T. H. Steenkiste, J. R. Smith and R. E. Teets, "Aluminum coatings via kinetic spray with relatively large powder particles," *Surface and Coatings Technology*, vol. 154, pp. 237-252, 2002.

[13] G. Bae, Y. Xiong, S. Kumar, K. Kang and C. Lee, "General aspects of interface bonding in kinetic sprayed coatings," *Acta Materialia*, vol. 56, pp. 4858-4868, 2008.

[14] S. Yin, X. Wang, W. Li, H. Liao and H. Jie, "Deformation behavior of the oxide film on the surface of cold sprayed powder particle," *Applied Surface Science*, vol. 259, pp. 294-300, 2012.

[15] X. K. Wu, J. S. Zhang, X. L. Zhou, H. Cui and J. C. Liu, "Advanced cold spray technology: Deposition characteristics and potential applications," *Science China*, vol. 55, no. 2, pp. 357-368, 2012.

[16] T. Schmidt, H. Assadi, F. Gärtner, H. Richter, T. Stoltenhoff, H. Kreye and T. Klassen, "From Particle Acceleration to Impact and Bonding in Cold Spraying," *Journal of Thermal Spray Technology*, vol. 18, no. 5-6, pp. 794-808, 2009.

[17] M. Grujicic, C. L. Zhao, C. Tong, W. S. DeRosset and D. Helfritch, "Analysis of the impact velocity of powder particles in the cold-gas dynamic-spray process," *Materials Science and Engineering A*, vol. 368, pp. 222-230, 2004.

[18] T. Schmidt, F. Gaertner, H. Assadi and H. Kreye, "Development of a generalized parameter window for cold spray deposition," *Acta Materialia*, vol. 54, no. 3, pp. 729-742, 2006.

[19] A. Ganesan, J. Affi, M. Yamada and M. Fukumoto, "Bonding behavior studies of cold sprayed copper coating on the PVC," *Surface & Coatings Technology*, vol. 207, pp. 262-269, 2012.

[20] J. H. Lee, H. L. Jang, K. M. Lee, H.-R.Baek, K. Jin, K. S. Hong, J. H. Noh and H.-K. Lee, "In vitro and in vivo evaluation of the bioactivity of hydroxyapatite-coated polyetherketone biocomposites created by cold spray technology," *Acta Materialia*, vol. 9, no. 4, pp. 6177-6187, 2013.

[21] F. J. Brodmann, “Cold spray process parameters: powders,” in *The cold spray materials deposition process*, Cambridge, Woodhead Publishing Limited, 2007, pp. 105-116.

[22] H. Koivuluoto, G. Bolelli, L. Lusvarghi, F. Casadei and P. Vuoristo, “Corrosion resistance of cold-sprayed Ta coatings in very aggressive conditions,” *Surface & Coatings Technology,* vol. 205, pp. 1103-1107, 2010.

[23] K. Kang, G. Bae, J. Won and C. Lee, “Mechanical property enhancement of kinetic sprayed Al coatings reinforced by multi-walled carbon nanotubes,” *Acta Materialia,* vol. 60, pp. 5031-5039, 2012.

[24] X. Zhou and P. Mohanty, “Electrochemical behavior of cold sprayed hydroxyapatite/titanium composite in Hanks' solution,” *Electrochimica Acta,* vol. 65, pp. 134-140, 2012.

[25] N. T. Salim, M. Yamada, H. Isago, K. Shima, H. Nakano and M. Fukumoto, “The understanding on adhesion mechanism of cold sprayed TiO2 coating,” in *Proceedings of the International Thermal Spray Conference,* Hamburg, 2011.

[26] A. Trentin, S. Vezzu, S. Rech, S. Gulizia and M. Jahedi, “Biocompatibility of titanium coatings deposites on CoCr by cold spray,” in *Proceedings of the International Thermal Spray Conference,* Hamburg, 2011.

[27] J. Cizek, O. Kovarik, J. Siegl, K. A. Khor and I. Dlouhy, “Influence of plasma and cold spray deposited Ti layers on high-cycle fatigue properties of Ti6Al4V substrates,” *Surface & Coatings Technology,* vol. 217, pp. 23-33, 2013.

[28] T. Hussain, D. G. McCartney and P. H. Shipway, “Impact phenomena in cold-spraying of titanium onto verious ferrous alloys,” *Surface & Coatings Technology,* vol. 205, pp. 5021-5027, 2011.

[29] P. Vo, E. Irissou, J. -G. Legoux and S. Yue, “Mechanical and Microstructural Characterization of Cold-Sprayed Ti-6Al-4V After Heat Treatment,” *Journal of Thermal Spray Technology,* 2013.

[30] R. Lupoi and W. O'Neill, “Deposition of metallic coatings on polymer surfaces using cold spray,” *Surface & Coatings Technology,* vol. 205, pp. 2167-2173, 2010.

[31] P. C. King, A. J. Poole, S. Horne, R. d. Nys, S. Gulizia and M. Z. Jahedi, "Embedment of copper particles into polymers by cold spray," *Surface & Coatings Technology,* vol. 216, pp. 60-67, 2013.

[32] F. Robitaille, M. Yandouzi, S. Hind and B. Jodoin, "Metallic coating of aerospace carbon/epoxy composites by the pulsed gas dynamic spraying process," *Surface & Coatings Technology,* vol. 203, pp. 2954-2960, 2009.

[33] X. L. Zhou, A. Chen, J. Liu, X. K. Wu and J. S. Zhang, "Preparation of metallic coatings on polymer matrix composites by cold spray," *Surface & Coatings Technology,* vol. 206, pp. 132-136, 2011.

[34] D. Rafaja, T. Schucknecht, V. Klemm, A. Paul and H. Berek, "Microstructural characterisation of titanium coatings deposited using cold gas spraying on Al2O3 substrates," *Surface & Coatings Technology,* vol. 203, pp. 3206-3213, 2009.

[35] P. C. King, S. Zahiri, M. Jahedi and J. Friend, "Aluminium coating of lead zirconate titanate- A study of cold spray variables," *Surface & Coatings Technology,* vol. 205, pp. 2016-2022, 2010.

[36] E. Irissou, J.-G. Legoux, A. N. Ryabinin, B. Jodoin and C. Moreau, "Review on Cold Spray Process and Technology: Part I-Intellectual Property," *Journal of Thermal Spray Technology,* vol. 17, no. 4, pp. 495-516, 2008.

[37] J. Karthikeyan, "The advantages and disadvantages of the cold spray coating process," in *The cold spray materials deposition process,* Cambridge, Woodhead Publishing Limited, 2007, pp. 62-71.

[38] B. Al-Mangour, R. Dallala, F. Zhim, R. Mongrain and S. Yue, "Fatigue behavior of annealed cold-sprayed 316L stainless steel coating for biomedical applications," *Materials Letters,* vol. 91, pp. 352-355, 2013.

[39] X. Zhou and P. Mohanty, "Corrosion behaviour of cold sprayed titanium coatings in simulated body fluid," *Corrosion Engineering, Science and Technology,* vol. 47, no. 2, pp. 145-154, 2012.

[40] B. Al-Mangour, R. Mongrain, E. Irissou and S. Yue, "Improving the strength and corrosion resistance of 316L stainless steel for biomedical application using cold spray," *Surface & Coatings Technonology,* vol. 216, pp. 297-307, 2013.

[41] S. Sampath, X. Y. Jiang, J. Matejicek, L. Prchlik, A. Kulkarni and A. Vaidya, "Role of thermal spray processing method on the microstructure, residual stress and properties of coatings: an integrated study for Ni-5 wt.%Al bond coats," *Materials Science and Engineering A,* vol. 364, pp. 216-231, 2004.

[42] M. F. Smith, "Comparing cold spray with thermal spray coating technologies," in *The cold spray materials deposition process,* Cambridge, Woodhead Publishing Limited, 2007, pp. 43-61.

[43] M. Niinomi, "Metallic biomaterials," *Journal of Artificial Organs,* vol. 11, pp. 105-110, 2008.

[44] M. Niinomi, M. Nakai and J. Hieda, "Development of new metallic alloys for biomedical applications," *Acta Biomaterialia,* vol. 8, pp. 3888-3903, 2012.

[45] R. Pilliar and S. D. Ramsay, "Cobalt-Base Alloys," in *ASM Handbook: Materials for Medical Devices,* ASM International, 2012, pp. 211-222.

[46] P. Gill, N. Munroe, C. Pulletikurthi, S. Pandya and W. Halder, "Effect of Manufacturing Process on the Biocompatibility and Mechanical Properties of Ti-30Ta Alloy," *Journal of Materials Engineering and Performance,* vol. 20, no. 4-5, pp. 819-823, 2011.

[47] M. Niinomi, "Mechanical biocompatibilities of titanium alloys for biomedical applications," *Journal of the Mechanical Behavior of Biomedical Materials I,* pp. 30-42, 2008.

[48] M. A.-H. Gepreel and M. Niinomi, "Biocompatibility of Ti-alloys for long-term implantation," *Journal of the Mechanical Behaviour of Biomedical Materials,* vol. 20, pp. 407-415, 2013.

[49] T. Hanawa, "Research and development of metals for medical devices based on clinical needs," *Science and Technology of Advanced Materials,* vol. 13, pp. 1-15, 2012.

[50] T. S. Price, P. H. Shipway and D. G. McCartney, "Effect of Cold Spray Deposition of a Titanium Coating on Fatigue Behavior of a Titanium Alloy," *Journal of Thermal Spray Technology,* vol. 15, no. 4, pp. 507-512, 2006.

[51] H. Li, K. A. Khor and P. Cheang, "Adhesive and bending failure of thermal sprayed hydroxyapatite coatings: Effect of nanostructures at interface and crack propagation phenomenon during bending," *Engineering Fracture Mechanics,* vol. 74, pp. 1894-1903, 2007.

[52] R. S. Lima, H. Li, K. A. Khor and B. R. Marple, "Biocompatible Nanostructured High-Velocity Oxyfuel Sprayed Titania Coating: Deposition, Characterization, and Mechanical Properties," *Journal of Thermal Spray Technology,* vol. 15, no. 4, pp. 623-627, 2006.

[53] T. Laonapakul, A. R. Nimkerdphol, Y. Otsuka and Y. Mutoh, "Failure behavior of plasma-sprayed HAp coating on commercially pure titanium substrate in simulated body fluid (SBF) under bending load," *Journal of the mechanical behavior of biomedical materials,* vol. 15, pp. 153-166, 2012.

[54] T. Laonapakul, Y. Otsuka, A. R. Nimkerdphol and Y. Mutoh, "Acoustic emission and fatigue damage induced in plasma-sprayed hydroxyapatite coating layers," *Journal of the Mechanical Behaviour of Biomedical Materials,* vol. 8, pp. 123-133, 2012.

[55] X. Zhou, R. Siman, L. Lu and P. Mohanty, "Argon atmospheric plasma sprayed hydroxyapatite/Ti composite coating for biomedical applications," *Surface & Coatings Technology,* vol. 207, pp. 343-349, 2012.

[56] Y. W. Gu, K. A. Khor and P. Cheang, "In vitro studies of plasma-sprayed hydroxyapatite/Ti-6Al-4V composite coatings in simulated body fluid," *Biomaterials,* vol. 24, pp. 1603-1611, 2003.

[57] S. Yugeswaran, C. P. Yoganand, A. Kobayashi, K. M. Paraskevopoulos and B. Subramanian, "Mechanical properties, electrochemical corrosion and in-vitro bioactivity of yttria stabilized zirconia reinforced hydroxyaoatite coatings prepared by gas tunnel type plasma spraying," *Journal of the Mechanical Behavior of Biomedical Materials,* vol. 9, pp. 22-33, 2012.

[58] F. Witte, "The history of biodegradable magnesium implants: A review," *Acta Biomaterialia,* vol. 6, pp. 1680-1692, 2010.

[59] M. P. Staiger, A. M. Pietak, J. Huadmai and G. Dias, "Magnesium and its alloys as orthopedic biomaterials: A review," *Biomaterials,* vol. 27, pp. 1728-1734, 2006.

[60] G. Wu, J. M. Ibrahim and P. K. Chu, "Surface design of biodegradable magnesium alloys - A review," *Surface & Coatings Technology,* vol. 233, pp. 2-12, 2013.

[61] H.Hornberger, S. Virtanen and A. R. Boccaccini, "Biomedical coatings on magnesium alloys - A review," *Acta Biomaterialia,* vol. 8, pp. 2442-2455, 2012.

[62] A. C. W. Noorakma, H. Zuhailawati, V. Aishvarya and B. K. Dhindaw, "Hydroxyapatite-Coated Magnesium-Based Biodegradable Alloy: Cold Spray Deposition and Simulated Body Fluid Studies," *Journal of Materials Engineering and Performance,* vol. 22, no. 10, pp. 2997-3004, 2013.

[63] L. Tan, X. Yu, P. Wan and K. Yang, "Biodegradable Materials for Bone Repairs: A Review," *Journal of Materials Science and Technology,* vol. 29, no. 6, pp. 503-513, 2013.

[64] Q. Wang, K. Spencer, N. Birbilis and M.-X. Zhang, "The influence of ceramic particles on bond strength of cold spray composite coatings on AZ91 alloy substrate," *Surface & Coatings Technology,* vol. 205, pp. 50-56, 2010.

[65] X. K. Suo, X. P. Guo, W. Y. Li, M. P. Planche and H. L. Liao, "Investigation of Deposition Behavior of Cold-Sprayed Magnesium Coating," *Journal of Thermal Spray Technology,* vol. 21, no. 5, pp. 831-837, 2012.

[66] X. K. Suo, M. Yu, W. Y. Li, M. P. Planche and H. L. Liao, "Effect of Substrate Preheating on Bonding Strength of Cold-Sprayed Mg Coatings," *Journal of Thermal Spray Technology,* vol. 21, no. 5, pp. 1091-1098, 2012.

[67] D. M. Findlay, K. Welldon, G. J. Atkins, D. W. Howie, A. C. W. Zannettino and D. Bodyn, "The proliferation and phenotypic expression of human osteoblasts on tantalum metal," *Biomaterials,* vol. 25, pp. 2215-2227, 2004.

[68] Z. Tang, Y. Xie, F. Yang, Y. Huang, C. Wang, K. Dai, X. Zheng and X. Zhang, "Porous Tantalum Coatings Prepared by Vacuum Plasma Spraying Enhance BMSCs Osteogenic Differentiation and Bone Regeneration In Vitro and In Vivo," *PLOS ONE,* vol. 8, no. 6, pp. 1-11, 2013.

[69] N. Wang, H. Li, J. Wang, S. Chen, Y. Ma and Z. Zhang, "Study on the Anticorrosion, Biocompatibility, and Osteoinductivity of Tantalum Decorated with

Tantalum Oxide Nanotube Array Films," *ACS Applied Materials & Interfaces,* vol. 4, pp. 4516-4523, 2012.

[70] E. Kaivosoja, S. Myllymaa, Y. Takakubo, H. Korhonen, K. Myllymaa, Y. T. Konttinen, R. Lappalainen and M. Takagi, "Osteogenesis of human mesenchymal stem cells on micro-patterned surfaces," *Journal of Biomaterials Applications,* vol. 27, pp. 862-871, 2011.

[71] M. Stiehler, M. Lind, T. Mygind, A. Baatrup, A. Dolatshahi-Pirouz, H. Li, M. Foss, F. Besenbacher, M. Kassem and C. Bunger, "Morphology, proliferation, and osteogenic differentiation of mesenchymal stem cells cultured on titanium, tantalum, and chromium surfaces," *Journal of Biomedical Materials Research Part A,* vol. 86, no. 2, pp. 448-458, 2008.

[72] G. Bolelli, B. Bonferroni, H. Koivuluoto, L. Lusvarghi and P. Vuoristo, "Depth-sensing indentation for assessing the mechanical properties of cold-sprayed Ta," *Surface & Coatings Technology,* vol. 205, pp. 2209-2217, 2010.

[73] E. Bannier, G. Darut, E. Sanchez, A. Denoirjean, M. C. Bordes, M. D. Salvador, E. Rayon and H. Ageorges, "Microstructure and photocatalytic activity of suspension plasma sprayed TiO2 coatings on steel and glass substrates," *Surface & Coatings Technology,* vol. 206, pp. 378-386, 2011.

[74] A. Simchi, E. Tamjid, F. Pishbin and A. R. Boccaccini, "Recent progress in inorganic and composite coatings with bactericidal capability for orthopaedic applications," *Nanomedicine: Nanotechnology, Biology and Medicine,* vol. 7, pp. 22-39, 2011.

[75] N. T. Salim, M. Yamada, H. Nakano, H. Isago, K. Shima and M. Fukumoto, "The synthesis of titanium dioxide powders for cold spray," in *Proceedings of the International Thermal Spray Conference*, Hamburg, 2011.

[76] J.-O. Kliemann, H. Gutzmann, F. Gärtner, H. Hubner, C. Borchers and T. Klassen, "Formation of Cold-Sprayed Ceramic Titanium Dioxide Layers on Metal Surfaces," *Journal of Thermal Spray Technology,* vol. 20, no. 1-2, pp. 292-298, 2010.

[77] H. Melero, J. Fernandez, S. Dosta and J. M. Guilemany, "Characterization of novel bioactive hydroxyapatite-TiO2 coatings obtained by high velocity oxy-fuel," in *Proceedings of the Thermal Spray Conference*, Hamburg, Germany, 2011.

[78] A. Ibrahim, R. S. Lima, C. C. Berndt and B. R. Marple, “Fatigue and mechanical properties of nanostructured and conventional titania (TiO2) thermal spray coatings,” *Surface & Coatings Technology,* vol. 201, pp. 7589-7596, 2007.

[79] M. Gaona, R. S. Lima and B. R. Marple, “Nanostructured titania/hydroxyapatite composite coatings deposited by high velocity oxy-fuel (HVOF) spraying,” *Materials Science and Engineering A,* vol. 458, pp. 141-149, 2007.

[80] J. -G. Legoux, F. Chellat, R. S. Lima, B. R. Marple, M. N. Bureau, H. Shen and G. A. Candeliere, “Development of Osteoblast Colonies on New Bioactive Coatings,” *Journal of Thermal Spray Technology,* vol. 15, no. 4, pp. 628-633, 2006.

[81] S. Bauer, P. Schmuki, K. v. d. Mark and J. Park, “Engineering biocompatible implant surfaces Part I: Materials and surfaces,” *Progress in Materials Science,* vol. 58, pp. 261-326, 2013.

[82] A. Osaka, “Ceramic Materials,” in *ASM Handbook, Materials for Medical Devices Vol. 23,* ASM International, 2012, pp. 265-277.

[83] G. Wang, X. Liu, J. Gao and C. Ding, “In vitro bioactivity and phase stability of plasma-sprayed nanostructured 3Y-TZP coatings,” *Acta Biomaterialia,* vol. 5, pp. 2270-2278, 2009.

[84] H. C. Gledhill, I. G. Turner and C. Doyle, “In vitro fatigue behaviour of vacuum plasma and detonation gun sprayed hydroxyapatite coatings,” *Biomaterials,* vol. 22, pp. 1233-1240, 2001.

[85] R. B. Heimann, “Structure, properties, and biomedical performance of osteoconductive bioceramic coatings,” *Surface & Coatings Technology,* vol. 233, pp. 27-38, 2013.

[86] R. Gadow, A. Killinger and N. Stiegler, “Hydroxyapatite coatings for biomedical applications deposited by different thermal spray techniques,” *Surface & Coatings Technology,* vol. 205, pp. 1157-1164, 2010.

[87] G. Bolelli, N. Stiegler, D. Bellucci, V. Cannillo, R. Gadow, A. Killinger, L. Lusvarghi and A. Sola, “Deposition mechanisms in high velocity suspension spraying: Case study for two bioactive materials,” *Surface & Coatings Technology,* vol. 210, pp. 28-45, 2012.

[88] H. Podlesak, L. Pawlowski, R. d'Haese, J. Laureyns, T. Lampke and S. Bellayer, "Advanced Microstructural Study of Suspension Plasma Sprayed Hydroxyapatite Coatings," *Journal of Thermal Spray Technology,* vol. 19, no. 3, pp. 657-664, 2010.

[89] L. Latka, L. Pawlowski, D. Chicot, C. Pierlot and F. Petit, "Mechanical properties of suspension plasma sprayed hydroxyapatite coatings submitted to simulated body fluid," *Surface & Coatings Technology,* vol. 205, pp. 954-960, 2010.

[90] S. Kozerski, L. Pawlowski, R. Jaworski, F. Roudet and F. Petit, "Two zones microstructure of suspension plasma sprayed hydroxyapatite coatings," *Surface & Coatings Technology,* vol. 204, pp. 1380-1387, 2010.

[91] G. Bolelli, D. Bellucci, V. Cannillo, L. Lusvarghi, A. Sola, N. Stiegler, P. Muller, A. Killinger, R. Gadow, L. Altomare and L. D. Nardo, "Suspension thermal spraying of hydroxyapatite: Microstructure and in vitro behaviour," *Materials Science and Engineering C,* vol. 34, pp. 287-303, 2014.

[92] Y. Liu, Z. Dang, Y. Wang, J. Huang and H. Li, "Hydroxyapatite/graphene-nanosheet composite coatings deposited by vacuum cold spraying for biomedical applications: Inherited nanostructures," *Carbon,* vol. 67, pp. 250-259, 2014.

[93] G. Bolelli, V. Cannillo, R. Gadow, A. Killinger, L. Lusvarghi, A. Sola and N. Stiegler, "Microstructure and in-vitro behaviour of a novel High Velocity Suspension Flame Sprayed (HVSFS) bioactive glass coating," *Surface & Coatings Technology,* vol. 205, pp. 1145-1149, 2010.

[94] D. Bellucci, G. Bolelli, V. Cannillo, R. Gadow, A. Killinger, L. Lusvarghi, A. Sola and N. Stiegler, "High velocity suspension flame sprayed (HVSFS) potassium-based bioactive glass coatings with and without TiO2 bond coat," *Surface & Coatings Technology,* vol. 206, pp. 3857-3868, 2012.

[95] M. Monsalve, H. Ageorges, E. Lopez, F. Vargas and F. Bolivar, "Bioactivity and mechanical properties of plasma-sprayed coatings of bioglass powders," *Surface & Coatings Technology,* vol. 220, pp. 60-66, 2013.

[96] V. Cannillo, J. Colmenares-Angulo, L. Lusvarghi, F. Pierli and S. Sampath, "In vitro characterisation of plasma-sprayed apatite/wollastonite glass-ceramic biocoatings on titanium alloys," *Journal of the European Ceramic Society,* vol. 29, pp. 1665-1677, 2009.

[97] V. Cannillo and A. Sola, "Different approaches to produce coatings with bioactive glasses: Enamelling vs plasma spraying," *Journal of the European Ceramic Society*, vol. 30, pp. 2031-2039, 2010.

[98] A. Cattini, L. Latka, D. Bellucci, G. Bolelli, A. Sola, L. Lusvarghi, L. Pawlowski and V. Cannillo, "Suspension plasma sprayed bioactive glass coatings: Effects of processing on microstructure, mechanical properties and in-vitro behaviour," *Surface & Coatings Technology*, vol. 220, pp. 52-59, 2013.

[99] G. Wang, Z. Lu, X. Liu, X. Zhou, C. Ding and H. Zreiqat, "Nanostructured glass - ceramic coatings for orthopaedic applications," *Journal of the Royal Society Interface*, vol. 8, pp. 1192-1203, 2011.

[100] J. Yu, K. Li, X. Zheng, D. He, X. Ye and M. Wang, "In Vitro and In Vivo Evaluation of Zinc-Modified Ca-Si-Based Ceramic Coating for Bone Implants," *PLoS ONE*, vol. 8, no. 3, 2013.

[101] L. Qi, M. Li, S. Zhang, J. Xue and H. Si, "Comparative effectiveness of PEEK rods versus titanium alloy rods in lumbar fusion: A preliminary report," *Acta Neurochirurgica*, vol. 155, pp. 1187-1193, 2013.

[102] G. D. Schroeder, M. R. Murray and W. K. Hsu, "A Review of Dynamic Stabilization in the Lumbar Spine," *Operative Techniques in Orthopaedics*, pp. 235-239, 2011.

[103] R. K. Ponnappan, H. Serhan, B. Zarda, R. Patel, T. Albert and A. R. Vaccaro, "Biomedical evaluation and comparison of polyetheretherketone rod system to traditional titanium rod fixation," *The Spine Journal*, vol. 9, pp. 263-267, 2009.

[104] P. Robotti and G. Zappini, "Thermal Plasma Spray Deposition of Titanium and Hydroxyapatite on Polyaryletheretherketone Implants," in *PEEK Biomaterials Handbook*, Elsevier, 2012, pp. 119-142.

[105] M. Gardon, A. Latorre, M. Torrell, S. Dosta, J. Fernandez and J. M. Guilemany, "Cold gas spray titanium coatings onto a biocompatible polymer," *Materials Letters*, vol. 106, pp. 97-99, 2013.

[106] D. M. Devine, J. Hahn, R. G. Richards, H. Gruner, R. Wieling and S. G. Pearce, "Coating of carbon fiber-reinforced polyetheretherketone implants with titanium to

improve bone apposition," *Journal of Biomedical Materials Research -Part B Applied Biomaterials,* pp. 591-598, 2012.

[107] G. M. Wu, W. D. Hsiao and S. F. Kung, "Investigation of hydroxyapatite coated polyether ether ketone composites by gas plasma sprays," *Surface & Coatings Technology,* vol. 203, pp. 2755-2758, 2009.

[108] S. Beauvais and O. Decaux, "Plasma Sprayed Biocompatible Coatings on PEEK Implants," in *Thermal Spray 2007: Global Coating Solutions*, Materials Park, Ohio, USA, 2007.

[109] V. R. Sastri, "Engineering Thermoplastics: Acrylics, Polycarbonates, Polyurethanes, Polyacetals, Polyesters, and Polyamides," in *Plastics in Medical Devices*, Elsevier Inc., 2010, pp. 121-173.

[110] S. Lyu and D. Untereker, "Degradability of Polymers for Implantable Biomedical Devices," *International Journal of Molecular Sciences,* vol. 10, pp. 4033-4065, 2009.

[111] M. Grujicic, B. Pandurangan, W. C. Bell, M. Daqaq, L. Ma, N. Seyr, M. Ardmann and J. Holzleitner, "A computational analysis and suitability assessment of cold-gas dynamic spraying of glass-fiber-reinforced poly-amide 6 for use in direct-adhesion polymer metal hybrid components," *Applied Surface Science,* vol. 254, pp. 2136-2145, 2008.

[112] C. Auclair-Daigle, M. N. Bureau, J.-G. Legoux and L. Yahia, "Bioactive hydroxyapatite coatings on polymer composites for orthopedic implants," *Journal of Biomedical Materials Research-Part A,* vol. 73, no. 4, pp. 398-408, 2005.

[113] S. Hacking, T. Pauyo, L. Lim, J. G. Legoux and M. N. Bureau, "Tissue response to the components of a hydroxyapatite-coated composite femoral implant," *Journal of Biomedical Materials Research -Part A,* vol. 94, no. 3, pp. 953-960, 2010.

[114] E. Gomez-Barrera, J.-A. Puertolas, L. Munuera and Y. T. Konttinen, "Update on UHMWPE research," *Acta Orthopaedica,* vol. 79, no. 6, pp. 832-840, 2008.

[115] A. Chebbi, J. Podporska and J. Stokes, "Thermal spraying of bioactive polymer coatings for orthopaedic applications," in *Thermal Spray 2011: Proceedings of the International Thermal Spray Conference*, Hamburg, Germany, 2011.

[116] A. Chebbi, "Doctoral Dissertation: Thermal Spray of a Drug Delivery System onto Femoral Orthopaedic Implants," School of Mechanical and Manufacturing Engineering, Dublin City Univeristy, Ireland, 2011.

[117] L. T. Kuhn, "Biomaterials," in *Introduction to biomedical engineering*, Connecticut, Elsevier, 2012, pp. 219-271.

[118] M. H. Ross and W. Pawlina, Histology, Wolters Kluwer/Lippincott Williams & Wilkins, 2011.

[119] J. E. Hall and A. C. Guyton, "Acid-Base Regulation," in *Medical Physiology*, Elsevier, 2011, pp. 379-396.

[120] J. M. Anderson, A. Rodriguez and D. T. Chang, "Foreign body reaction to biomaterials," *Seminars in Immunology*, vol. 20, pp. 86-100, 2008.

[121] J. E. Hall and A. C. Guyton, "The Body Fluid Compartments: Extracellular and Intracellular Fluids; Edema," in *Medical Physiology*, Elsevier, 2011, pp. 285-301.

[122] D. Shi, Biomaterials and Tissue Engineering, Berlin, Germany: Springer, 2004.

[123] M. H. Ross and W. Pawlina, "Cartilage," in *Histology*, Wolters Kluwer/ Lippicott Williams & Wilkins, 2011, pp. 198-217.

[124] M. H. Ross and W. Pawlina, "Bone," in *Histology*, Wolters Kluwer/ Lippicott Williams & Wilkins, 2011, pp. 218-253.

[125] B. K. Hall, "Types of Skeletal Tissues," in *Bones and Cartilage: Developmental and Evolutionary Skeletal Biology*, U.S.A., Elsevier Ltd., 2005, pp. 3-12.

[126] G. A. Holzapfel, "Biomechanics of Soft Tissue," in *Handbook of Material Behaviour: Nonlinear Models and Properties*, 2000.

[127] B. Alberts, D. Bray, K. Hopkin, A. Johnson, J. Lewis, M. Raff, K. Roberts and P. Walter, Essential Cell Biology, Garland Science, 2004.

[128] K. v. d. Mark and J. Park, "Engineering biocompatible surfaces Part II: Cellular recognition of biomaterial surfaces: Lessons from cell-matrix interactions," *Progress in Materials Science*, vol. 58, pp. 327-381, 2013.

[129] P. Schaffner and M. M. Dard, "Structure and function of RGD peptides involved in bone biology," *Cellular and Molecular Life Sciences,* vol. 60, pp. 119-132, 2003.

[130] J. J. Ramsden, D. M. Allen, D. Stephenson, J. R. Alcock, G. N. Peggs, G. Fuller and G. Goch, "The Design and Manufacture of Biomedical Surfaces," *Annals of the CIRP,* vol. 56, pp. 687-711, 2007.

[131] D. F. Williams, "On the mechanisms of biocompatibility," *Biomaterials,* vol. 29, pp. 2941-2953, 2008.

[132] J. Satulovsky, M. A. Carignano and I. Szleifer, "Kinetic and thermodynamic control of protein adsorption," *Proceedings of the National Academy of Sciences,* vol. 97, no. 16, pp. 9037-9041, 2000.

[133] K. Nakanishi, T. Sakiyama and K. Imamura, "On the Adsorption of Proteins on Solid Surfaces, a Common but Very Complicated Phenomenon," *Journal of Bioscience and Bioengineering,* vol. 91, no. 3, pp. 233-244, 2001.

[134] R. A. Hartvig, M. v. d. Weert, J. Østergaard, L. Jorgensen and H. Jensen, "Protein Adsorption at Charged Surfaces: The Role of Electrostatic Interactions and Interfacial Charge Regulation," *Langmuir,* vol. 27, pp. 2634-2643, 2011.

[135] P. Thevenot, W. Hu and L. Tang, "Surface chemistry influence implant biocompatibility," *Current Topics in Medicinal Chemistry,* vol. 8, no. 4, pp. 270-280, 2008.

[136] A. Krishnan, C. A. Siedlecki and E. A. Vogler, "Mixology of Protein Solution and the Vroman Effect," *Langmuir,* vol. 20, pp. 5071-5078, 2004.

[137] S.-Y. Jung, S.-M. Lim, F. Albertorio, G. Kim, M. C. Gurau, R. D. Yang, M. A. Holden and P. S. Cremer, "The Vroman Effect: A Molecular Level Description of Fibrinogen Displacement," *Journal of American Chemical Society,* vol. 125, pp. 12782-12786, 2003.

[138] E. A. Volger, "Protein Adsorption in Three Dimensions," *Biomaterials,* vol. 33, no. 5, pp. 1201-1237, 2012.

[139] L. Tang and J. W. Eaton, "Natural Responses to Unnatural Materials: A Molecular Mechanism for Foreign Body Reactions," *Molecular Medicine,* vol. 5, pp. 351-358, 1999.

[140] B. Rolfe, J. Mooney, B. Zhang, S. Jahnke, S.-J. Le, Y.-Q. Chau, Q. Huang, H. Wang, G. Campbell and J. Campbell, "The Fibrotic Response to Implanted Biomaterial: Implications for Tissue Engineering," in *Regenerative Medicine and Tissue Engineering-Cells and Biomaterials*, Rijeka, InTech, 2011, pp. 551-568.

[141] E. Song, N. Ouyang, M. Hörbelt, B. Antus, M. Wang and M. S. Exton, "Influence of Alternatively and Classically Activated Macrophages on Fibrogenic Activities of Human Fibroblasts," *Cellular Immunology,* vol. 204, pp. 19-28, 2000.

[142] J. M. S. Zeller, "Surgical Implants: Physiological Response," *AORN Journal,* vol. 37, no. 7, pp. 1284-1291, 1983.

[143] J. M. Bryers, "Medical Biofilms," *Biotechnology and Bioengineering,* vol. 100, no. 1, pp. 1-18, 2008.

[144] M. T. Novak, J. D. Bryers and W. M. Reichert, "Biomimetic strategies based on viruses and bacteria for the development of immune evasive biomaterials," *Biomaterials,* vol. 30, pp. 1989-2005, 2009.

[145] D. Arola, D. Bajaj, J. Ivancik, H. Majd and D. Zhang, "Fatigue of biomaterials: Hard tissues," *International Journal of Fatigue,* vol. 32, pp. 1400-1412, 2010.

[146] "General Materials Data," in *ASM Desk Editions,* ASM International, 1995.

[147] J. W. Nicholson, The Chemistry of Medical and Dental Materials, Cambridge, UK: The royal society of chemistry, 2002.

[148] S. Lopez-Esteban, E. Saiz, S. Fujino, T. Oku, K. Suganuma and A. P. Tomsia, "Bioactive glass coatings for orthopedic metallic implants," *Journal of the European Ceramic Society,* vol. 23, pp. 2921-2930, 2003.

[149] Materials and coatings for medical devices: Cardiovascular, ASM International, 2009.

[150] T. Okamoto, M. Neo, S. Fujibayashi, H. Ito, M. Takemoto and T. Nakamura, "Mechanical implant failure in posterior cervical spine fusion," *European Spine Journal,* vol. 21, pp. 328-334, 2012.

[151] L. Pruitt and J. Furmanski, "Polymeric biomaterials for load-bearing medical devices," *JOM,* vol. 61, no. 9, pp. 14-20, 2009.

[152] F. Gärtner, T. Stoltenhoff, J. Voyer, H. Kreye, S. Riekehr and M. Kocak, "Mechanical properties of cold-sprayed and thermally sprayed copper coatings," *Surface & Coatings Technology,* vol. 200, pp. 6770-6782, 2006.

[153] R. A. Antunes and M. C. L. d. Oliveira, "Corrosion fatigue of biomedical metallic alloys: Mechanisms and mitigation," *Acta Biomaterialia,* vol. 8, pp. 937-962, 2012.

[154] S. H. Teoh, "Fatigue of biomaterials: a review," *International Journal of Fatigue,* vol. 22, pp. 825-837, 2000.

[155] S. J. Shaffer and W. A. Glaeser, "Fretting and Fretting Fatigue Mechanisms," in *ASM Handbook Vol 8.,* ASM International, 2000, pp. 730-739.

[156] R. Ahmed and M. Hadfield, "Mechanisms of Fatigue Failure in Thermal Spray Coatings," *Journal of Thermal Spray Technology,* vol. 11, no. 3, pp. 333-349, 2002.

[157] L. Lapaj, J. Markuszewski, T. Rybak and M. Wierusz-Kozlowska, "Debonding of porous of a threaded acetabular component: Retrieval analysis," *Journal of the mechanical behaviour of biomedical materials,* vol. 17, pp. 107-111, 2013.

[158] M. A. Jacobs, T. Bhargava, J. M. Lathroum and M. Hungerford, "Debonding of the Acetabular Porous Coating in Hip Resurfacing Arthroplasty," *The Journal of Bone & Joint Surgery,* Vols. 91-A, no. 4, pp. 961-964, 2009.

[159] H. P. Delport, B. V. Backle and J. D. Schepper, "Debonding of the acetabular porous coating in hip resurfacing arthroplasty: A case report," *Acta Orthopaedica Belgica,* vol. 77, pp. 125-127, 2011.

[160] P. Ylinen, "Acetabular cup loosening-peeling off of the plasma-sprayed porous coating in 2 cases," *Acta Orthopaedica Scandinavica,* vol. 67, no. 5, pp. 508-512, 1996.

[161] E. Sansoucy, G. E. Kim, A. L. Moran and B. Jodoin, "Mechanical Characteristics of Al-Co-Ce Coatings Produced by the Cold Spray Process," *Journal of Thermal Spray Technology,* vol. 16, no. 5-6, pp. 651-660, 2007.

[162] A. Choudhuri, P. S. Mohanty and J. Karthikeyan, "Bio-ceramic Composite Coatings by Cold Spray Technology," in *Thermal Spray 2009: Proceedings of the International Thermal Spray Conference*, Las Vegas, USA, 2009.

[163] A. Ferrer, I. Garcia, J. Fernandez and J. M. Guilemany, "Study of Adhesion Relationship of Hydroxyapatite-Titania Coating Obtained by HVOF," *Materials Science Forum,* Vols. 636-637, pp. 82-88, 2010.

[164] D. Qiu, M. Zhang and L. Groendahl, "A novel composite porous coating approach for bioactive titanium-based orthopedic implants," *Journal of Biomedical Materials Research A,* vol. 00A, pp. 1-11, 2012.

[165] J. Sun, Y. Han and K. Cui, "Innovative fabrication of porous titanium coating on titanium by cold spraying and vacuum sintering," *Materials Letters,* vol. 62, pp. 3623-3625, 2008.

[166] K. Binder, J. Gottschalk, M. Kollenda, F. Gärtner and T. Klassen, "Influence of Impact Angle and Gas Temperature on Mechanical Properties of Titanium Cold Spray Deposits," *Journal of Thermal Spray Technology,* vol. 20, no. 1-2, pp. 234-242, 2011.

[167] I. Burlacov, J. Jirkovsky, L. Kavan, R. Ballhorn and R. B. Heimann, "Cold gas dynamic spraying (CGDS) of TiO2 (anatase) powders onto poly(sulfone) substrates: Microstructural characterisation and photocatalytic efficiency," *Journal of Photochemistry and Photobiology A: Chemistry,* vol. 187, pp. 285-292, 2007.

[168] K. Sano, K. Ito and K. Yamamoto, "Changes of bone mineral density after cementless total hip arthroplasty with two different stems," *International Orthopaedics,* vol. 32, pp. 167-172, 2008.

[169] K. Kawate, Y. Ohneda, T. Ohmura, H. .. Yajima, K. Sugimoto and Y. Takakura, "Computed Tomography–Based Custom-Made Stem for Dysplastic Hips in Japanese Patients," *The Journal of Arthroplasty,* vol. 24, no. 1, pp. 65-70, 2009.

[170] A. Skardal, D. Mack, A. Atala and S. Soker, "Substrate elasticity controls sell proliferation, surface marker expression and motile phenotype in amniotic fluid-

derived stem cells," *Journal of the Mechanical Behaviour of Biomedical Materials,* vol. 17, pp. 307-316, 2013.

[171] A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher, "Matrix Elasticity Directs Stem Cell Lineage Specification," *Cell,* vol. 126, no. 4, pp. 677-689, 2006.

[172] X. Zhao, M. Niinomi, M. Nakai and J. Hieda, "Beta type Ti-Mo alloys with changeable Young's modulus dor spinal fixation applications," *Acta Biomaterialia,* vol. 8, pp. 1990-1997, 2012.

[173] M. Niinomi and M. Nakai, "Titanium-Based Biomaterials for Preventing Stress Shielding between Implant Devices and Bone," *Biomaterials,* vol. Article ID 836587, pp. 1-10, 2011.

[174] S. M. Kurtz and J. N. Devine, "PEEK biomaterials in trauma, orthopaedic, and spinal implants," *Biomaterials,* vol. 28, pp. 4845-4869, 2007.

[175] M. A. Freilich and J. C. Meiers, "Fiber-reinforced composite prostheses," *The Dental Clinics of North America,* vol. 48, pp. 545-562, 2004.

[176] J.-L. Sui, M.-S. Li, Y.-P. Lu, L.-W. Yin and Y.-J. Song, "Plasma-sprayed hydroxyapatite coatings on carbon/carbon composites," *Surface & Coatings Technology,* vol. 176, pp. 188-192, 2004.

[177] S. V. Raj, R. Pawlik and W. Loewenthal, "Young's modulus of cold and vacuum plasma sprayed metallic coatings," *Materials Science and Engineering A,* Vols. 513-514, pp. 59-63, 2009.

[178] J. Schrooten, G. Roebben and J. A. Helsen, "Young's modulus of bioactive glass coated oral implants: porosity corrected bulk modulus versus resonance frequency analysis," *Scripta Materialia,* vol. 41, no. 10, pp. 1047-1053, 1999.

[179] Y. Chen, S. R. Bakshi and A. Agarwal, "Correlation between nanoindentation and nanoscratch properties of carbon nanotube reinforced aluminum composite coatings," *Surface & Coatings Technology,* vol. 204, pp. 2709-2715, 2010.

[180] M. Geetha, A. K. Singh, R. Asokamani and A. K. Gogia, "Ti based biomaterials, the ultimate choice for orthopaedic implants - A review," *Progress in Materials Science,* vol. 54, pp. 397-425, 2009.

[181] W. D. Callister, "Corrosion and Degradation of Materials," in *Materials Science and Engineering: An Introduction sixth edition*, J. Wiley & Sons Inc., 2003, pp. 569-610.

[182] H. Koivuluoto, Microstructural Characteristics and Corrosion Properties of Cold-Sprayed Coatings, Tampere University of Technology: Doctor's Dissertation, 2010.

[183] P. Elliott, "Selecting Materials to Prevent or Control Corrosion," in *ASM Handbook, Materials Selection for Corrosion Control, Corrosion: Fundamentals, Testing, and Protection, Vol 13A*, ASM International, 2003, pp. 909-928.

[184] C. Tkaczyk and M. Tabrizian, "Biocompatibility, Metal Ions, and Corrosion Products," in *Materials for Medical Devices, Vol 23, ASM Handbook*, ASM International, 2012, pp. 47-55.

[185] K. R. S. John, "Biocompatiblity of Metallic Materials for Medical Devices -The Effects of Corrosion and Corrosion Products," in *Materials for Medical Devices, Vol 23, ASM Handbook,* ASM International, 2012, pp. 73-78.

[186] D. Dzhurinskiy, E. Maeva, E. Leshchinsky and R. G. Maev, "Corrosion Protection of Light Alloys Using Low Pressure Cold Spray," *Journal of Thermal Spray Technology,* vol. 21, no. 2, pp. 304-313, 2012.

[187] F. Witte, N. Hort, C. Vogt, S. Cohen, K. U. Kainer, R. Willumeit and F. Feyerabend, "Degradable biomaterials based on magnesium corrosion," *Current Opinion in Solid State and Materials Science,* vol. 12, pp. 63-72, 2008.

[188] L. Grillini and S. Affatato, "How to measure wear following total hip arthtoplasty," *Hip International,* vol. 23, no. 3, pp. 233-242, 2013.

[189] E. Ingham and J. Fisher, "Biological reactions to wear debris in total joint replacement," *Proceedings of the Institution of Mechanical Engineerings, Part H: Journal of Engineering in Medicine,* vol. 214, pp. 21-37, 2000.

[190] R. B. Martin, D. B. Burr and N. A. Sharkey, Skeletal tissue mechanics, New York: Springer Verlag, 1998.

[191] T. M. McCloughlin and A. G. Kavanagh, "Wear of ultra-high molecular weight polyethylene (UHMWPE) in total knee prostheses: A review of key influences,"

Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine, vol. 214, pp. 349-359, 2000.

[192] W. Jiang, H. Mashayekhi and B. Xing, "Bacterial toxicity comparison between nano- and micro-scaled oxide particles," *Environmental Pollution,* vol. 157, pp. 1619-1625, 2009.

[193] N. J. Hallab, "Biologic Aspects of Implant Wear," in *ASM handbook Vol. 23 Materials for Medical Devices,* ASM International, 2012, pp. 157-168.

[194] S. Sathish, M. Geetha, S. T. Aruna, N. Balaji, K. S. Rajam and R. Asokamani, "Sliding wear behavior of plasma sprayed nanoceramic coatings for biomedical applications," *Wear,* vol. 271, pp. 934-941, 2011.

[195] M. F. Morks, N. F. Fahim and A. Kobayashi, "Structure, mechanical performance and electrochemical characterization of plasma sprayed SiO2/Ti-reinforced hydroxyapatite biomedical coatings," *Applied Surface Science,* vol. 255, pp. 3426-3433, 2008.

[196] S. Nag and R. Banerjee, "Fundamentals of Medical Implant Materials," in *Materials for Medical Devices,* ASM International, 2012, pp. 6-17.

[197] W. Hofstetter, H. Sehr, M. d. Wild, J. Portenier, J. Gobrecht and E. B. Hunziker, "Modulation of human osteoblasts by metal surface chemistry," *Journal of Biomedical Materials Research Part A,* vol. 101, no. 8, pp. 2355-2364, 2013.

[198] M. Roy, A. Bandyopadhyay and S. Bose, "Induction plasma sprayed nano hydroxyapatite coatings on titanium for orthopaedic and dental implants," *Surface & Coatings Technology,* vol. 205, pp. 2785-2792, 2011.

[199] R. S. Lima, S. Dimitrievska, M. N. Bureau, B. R. Marple, A. Petit, F. Mwale and J. Antoniou, "Enhanced Proliferation and Growth of Human Stem Cells on the Surface of HVOF-sprayed Nano TiO2-HA Coatings," in *Thermal Spray 2009: Proceedings of the International Thermal Spray Conference,* Las Vegas, 2009.

[200] H. Li and K. A. Khor, "Characteristics of the nanostructures in thermal sprayed hydroxyapatite coatings and their influence on coating properties," *Surface & Coatings Technology,* vol. 201, pp. 2147-2154, 2006.

[201] H. Kienapfel, C. Sprey, A. Wilke and P. Griss, “Implant Fixation by Bone Ingrowth,” *The Journal of Arthroplasty,* vol. 14, no. 3, pp. 355-368, 1999.

[202] D. A. Puleo and M. V. Thomas, “Implant Surfaces,” *The Dental Clinics of North America,* vol. 50, pp. 323-338, 2006.

[203] “Biomedical and Orthopedic Implants,” Ceramatec Inc., [Online]. Available: http://www.ceramatec.com/technology/more-ceramic-technologies/ceramatec-medical/biomedical-&-orthopedic-implants.php. [Accessed 8 1 2014].

[204] F. Yang, J. Lin, Y. He, H. Du and G. Chen, “Innovative fabrication of Ti-48Al-6Nb porous coating by cold gas spraying and reactive sintering,” *Materials Letters,* vol. 76, pp. 190-193, 2012.

[205] H. N. Kim, A. Jiao, N. Hwang, M. S. Kim, D. H. Kang, D.-H. Kim and K.-Y. Suh, “Nanotopography-guided tissue engineering and regenerative medicine,” *Advanced Drug Delivery Reviews,* vol. 65, pp. 536-558, 2013.

[206] K. Anselme, P. Davidson, A. M. Popa, M. Giazzon, M. Liley and L. Ploux, “The interaction of cells and bacteria with surfaces structured at the nanometre scale,” *Acta Biomaterialia,* vol. 6, pp. 3824-3846, 2010.

[207] X. Liu, P. K. Chu and C. Ding, “Surface nano-functionalization of biomaterials,” *Materials Science and Engineering R,* vol. 70, pp. 275-302, 2010.

[208] G. Zhao, A. L. Raines, M. Wieland, Z. Schwartz and B. D. Boyan, “Requirement for both micron- and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography,” *Biomaterials,* vol. 28, pp. 2821-2829, 2007.

[209] K. A. Gross, D. Muller, H. Lucas and D. R. Haynes, “Osteoclast resorption of thermal spray hydroxyapatite coatings is influenced by surface topography,” *Acta Biomaterialia,* vol. 8, pp. 1948-1956, 2012.

[210] G. Bae, K. Kang, J.-J. Kim and C. Lee, “Nanostructure formation and its effects on the mechanical properties of kinetic sprayed titanium coating,” *Materials Science and Engineering A,* vol. 527, pp. 6313-6319, 2010.

[211] L. Ajdelsztajn, B. Jodoin, G. E. Kim and J. M. Schoenung, "Cold spray deposition of nanocrystalline aluminum alloys," *Metallurgical and Materials Transactions A: Physical Metallurgy and Materials Science,* vol. 36, no. 3, pp. 657-666, 2005.

[212] L. Ajdelsztajn, B. Jodoin and J. M. Schoenung, "Synthesis and mechanical properties of nanocrystalline Ni coatings produced by cold gas dynamic spraying," *Surface and Coatings Technology,* vol. 201, no. 3-4, pp. 1166-1172, 2006.

[213] K. Mustafa, A. Wennerberg, J. Wroblewski, K. Hultenby, B. S. Lopez and K. Arvidson, "Determining optimal surface roughness of TiO2 blasted titanium implant material for attachment, proliferation and differentiation of cells derived from human mandibular alveolar bone," *Clinical Oral Implants Research,* vol. 12, pp. 515-525, 2001.

[214] D. Shtansky, I. V. Batenina, I. A. Yadroitsev, N. S. Ryashin, P. V. Kiryukhantsev-Korneev, A. E. Kudryashov, A. N. Sheveyko, I. Y. Zhitnyak, N. A. Gloushankova, I. Y. Smurov and E. A. Levashov, "A new combined approach to metal-ceramic implants with controllable surface topography, chemistry, blind porosity, and wettability," *Surface and Coatings Technology,* vol. 208, pp. 14-23, 2012.

[215] J. S. Hayes, J. L. Welton, R. Wieling and R. G. Richards, "In vivo evaluation of defined polished titanium surfaces to prevent soft tissue adhesion," *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* vol. 100B, no. 3, pp. 611-617, 2012.

[216] S. D. Puckett, E. Taylor, T. Raimondo and T. J. Webster, "The relationship between the nanostructure of titanium surfaces and bacterial attachment," *Biomaterials,* vol. 31, pp. 706-713, 2010.

[217] G. Yin, Z. Liu, J. Zhan, F. Ding and N. Yuan, "Impacts of the surface charge property on protein adsorption on hydroxyapatite," *Chemical Engineering Journal,* vol. 87, pp. 181-186, 2002.

[218] K. Wang, C. Zhou, Y. Hong and X. Zhang, "A review of protein adsorption on bioceramics," *Interface Focus,* vol. 2, pp. 259-277, 2012.

[219] S. Donatan, M. Sarikaya, C. Tamerler and M. Urgen, "Effect of solid surface charge on the binding behaviour of a metal-binding peptide," *Journal of Royal Society Interface,* vol. doi:10.1098/rsif.2012.0060, pp. 1-8, 2012.

[220] Y. Xie, H. Ao, S. Xin, X. Zheng and C. Ding, "Enhanced cellular responses to titanium coating with hierarchical hybrid structure," *Materials Science and Engineering C,* vol. 38, pp. 272-277, 2014.

[221] L. G. Harris and R. G. Richards, "Staphylicocci and implant surfaces: a review," *Injury, International Journal of the Care of the Injured,* vol. 37, pp. 3-14, 2006.

[222] G. Ren, D. Hu, E. W. C. Cheng, M. A. Vargas-Reus, P. Reip and R. P. Allaker, "Characterisation of copper oxide nanoparticles for antimicrobial applications," *International Journal of Antimicrobial Agents,* vol. 33, pp. 587-590, 2009.

[223] K. Li, Y. Xie, L. Huang, H. Ji and X. Zheng, "Antibacterial mechanism of plasma sprayed Ca2ZnSi2O7 coating against Escherichia coli," *Journal of Materials Science. Materials in Medicine,* vol. 24, pp. 171-178, 2013.

[224] N. Sanpo, S. M. Ang, P. Cheang and K. A. Khor, "Antibacterial Property of Cold Sprayed Chitosan-Cu/Al Coating," *Journal of Thermal Spray Technology,* vol. 18, no. 4, pp. 600-608, 2009.

[225] N. Sanpo, C. Hailan, K. Loke, K. P. Keng, P. Cheang, C. C. Berndt and K. A. Khor, "Biocompatibility and Antibacterial property of Cold Sprayed ZnO/Titanium Composite Coating," in *Science and Technology against microbial pathogens: Research, Development and Evaluation*, Valladolid, Spain, 2010.

[226] N. Sanpo, Saraswati, T. M. Lu and P. Cheang, "Anti-Bacterial Property of Cold Sprayed ZnO-Al Coating," in *Proceedings of the 2008 International Conference on BioMedical Engineering and Informatics,* Sanya, Hainan, China, 2008.

[227] N. Sanpo, M. L. Tan, P. Cheang and K. A. Khor, "Antibacterial Property of Cold-sprayed HA-Ag/PEEK Coating," *Journal of Thermal Spray Technology,* vol. 18, no. 1, pp. 10-15, 2009.

[228] K. Tamai, K. Kawate, I. Kawahara, Y. Takakura and K. Sakaki, "Inorganic antimicrobial coating for titanium alloy and its effect on bacteria," *Journal of Orthopaedic Science,* vol. 14, pp. 204-209, 2009.

[229] M. J. Vucko, P. C. King, A. J. Poole, C. Carl, M. Z. Jahedi and R. d. Nys, "Cold spray metal embedment: an innovative antifouling technology," *Biofouling,* vol. 28, no. 3, pp. 239-248, 2012.

[230] K. Li, Y. Xie, H. Ao, L. Huang, H. Ji and X. Zheng, "The enhanced bactericidal effect of plasma sprayed zinc-modified calcium silicate coating by the addition of silver," *Ceramics International,* vol. 39, pp. 7895-7902, 2013.

[231] B. Li, X. Liu, F. Meng, J. Chang and C. Ding, "Preparation and antibacterial properties of plasma sprayed nano-titania/silver coatings," *Materials Chemistry and Physics,* vol. 118, pp. 99-104, 2009.

[232] D. V. Dudina, S. B. Zlobin, V. Yu, N. V. Ulianitsky, N. V. Bulina, A. L. Bychkov and O. I. Lomovsky, "Detonation spraying of TiO2-Ag powders under a controllable atmosphere," in *Proceedings of the Thermal Spray Conference*, Hamburg, Germany, 2011.

[233] B. Li, X. Liu, C. Cao, Y. Dong and C. Ding, "Biological and Antibacterial Properties of Plasma Sprayed Wollastonite/Silver Coatings," *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* vol. 91, no. 2, pp. 596-603, 2009.

[234] T. Shimazaki, H. Miyamoto, Y. Ando, I. Noda, Y. Yonekura, S. Kawano, M. Miyazaki, M. Mawatari and T. Hotokebuchi, "In Vivo Antibacterial and Silver-Releasing Properties of Novel Thermal Sprayed Silver-Containing Hydroxyapatite Coating," *Journal of Biomedical Materials Research: B Applied Biomaterials,* vol. 92, no. 2, pp. 386-389, 2010.

[235] Y. Yonekura, H. Miyamoto, T. Shimazaki, Y. Ando, I. Noda, M. Mawatari and T. Hotokebuchi, "Osteoconductivity of thermal-sprayed silver-containing hydroxyapatite coating in the rat tibia," *The Journal of Bone & Joint Surgery (British edition),* vol. 93, pp. 644-649, 2011.

[236] G.-J. Yang, C.-J. Li, F. Han, W.-Y. Li and A. Ohmori, "Low temperature deposition and characterization of TiO2 photocatalytic film through cold spray," *Applied Surface Science,* vol. 254, pp. 3979-3982, 2008.

[237] I. Ivanova, J. Schneider, H. Gutzmann, J.-O. Kliemann, F. Gärtner, T. Klassen, D. Bahnemann and C. B. Mendive, "Photocatalytic degradation of oxalic and dichloroacetic acid on TiO2 coated metal substrates," *Catalysis Today,* vol. 209, pp. 84-90, 2013.

[238] S. Bose and S. Tarafder, “Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: A review,” *Acta Biomaterialia,* vol. 8, pp. 1401-1421, 2012.

[239] I. A. Karoussos, H. Wieneke, T. Sawitowski, S. Wnendt, A. Fischer, O. Dirsch, U. Dahmen and R. Erbel, “Inorganic materials as drug delivery systems in coronary artery stenting,” *Mat.-wiss. u. Werkstofftech.,* vol. 33, pp. 738-746, 2002.

[240] M. Taha, F. Chai, N. Blanchemain, M. Goube, B. Martel and H. F. Hildebrand, “Validating the poly-cyclodextrins based local drug delivery system on plasma-sprayed hydroxyapatite coated orthopedic implant with toluidine blue O,” *Materials Science and Engineering C,* vol. 33, pp. 2639-2647, 2013.

[241] B. Li, X. Liu, C. Cao and C. Ding, “Biocompatibility and antibacterial activity of plasma asprayed titania coating grafting collagen and gentamicin,” *Journal of Biomedical Materials Research A,* vol. 83, no. 4, pp. 923-930, 2007.

[242] B. Li, X. Liu, C. Cao, Y. Dong, Z. Wang and C. Ding, “Biological and antibacterial properties of plasma sprayed wollastonite coatings grafting gentamicin loaded collagen,” *Journal of Biomedical Materials Research A,* vol. 87, no. 1, pp. 84-90, 2008.

[243] A. Tan, Y. Farhatnia, A. d. Mel, J. Rajadas, M. S. Alavijeh and A. M. Seifalian, “Inception to actualization: Next generation coronary stent coatings incorporating nanotechnology,” *Journal of Biotechnology,* vol. 164, pp. 151-170, 2013.

[244] H. Schliephake, D. Scharnweber, M. Dard, A. Sewing, A. Aref and S. Roessler, “Functionalization of Dental Implant Surfaces Using Adhesion Molecules,” *Journal of Biomedical Material Research B: Applied Biomaterials,* vol. 73, no. 1, pp. 88-96, 2005.

[245] Y. Förster, C. Rentsch, W. Schneiders, R. Bernhardt, J. C. Simon, H. Worch and S. Rammelt, “Surface modification of implants in long bone,” *Landes Bioscience,* vol. 2, no. 3, pp. 149-157, 2012.

[246] V. Devescovi, E. Leonardi, G. Ciapetti and E. Cenni, “Growth factors in bone repair,” *La Chirurgia degli Organi di Movimento,* vol. 92, pp. 161-168, 2008.

[247] H. C. Tan, C. K. Poh, Y. Cai and W. Wang, "Anti-fibrosis effect of BMP-7 peptide functionalization on cobalt chromium alloy," *Journal of Orthopaedic Research,* vol. 31, no. 6, pp. 983-990, 2013.

[248] C. K. Poh, Z. Shi, X. W. Tan, Z. C. Liang, X. M. Foo, H. C. Tan, K. G. Neoh and W. Wang, "Cobalt chromium alloy with immobilized BMP peptide for enhanced bone growth.," *Journal of Orthopaedic Research,* vol. 29, no. 9, pp. 1424-1430, 2011.

[249] B. Peter, D. P. Pioletti, S. Laib, B. Bujoli, P. Pilet, P. Janvier, J. Guicheux, P.-Y. Zambelli, J.-M. Bouler and O. Gauthier, "Calcium phosphate drug delivery system: influence of local zoledronate release on bone implant osteointegration," *Bone,* vol. 36, pp. 52-60, 2005.

[250] T. Kokubo and H. Takadama, "How useful is SBF in predicting in vivo bone activity?," *Biomaterials,* vol. 27, pp. 2907-2915, 2006.

[251] H.-M. Kim, T. Himeno, T. Kokubo and T. Nakamura, "Process and kinetics of bonelike apatite formation on sintered hydroxyapatite in a simulated body fluid," *Biomaterials,* vol. 26, pp. 4366-4373, 2005.

[252] T. Cheng, Y. Chen and X. Nie, "Surface morphology manipulation and wear property of bioceramic oxide coatings on titanium alloy," *Surface & Coatings Technology,* vol. 215, pp. 253-259, 2013.

[253] Y. M. Thasneem and C. P. Sharma, "In Vitro Characterization of Cell-Biomaterials Interactions," in *Characterization of Biomaterials,* Elsevier Inc., 2013, pp. 175-205.

[254] T. L. Riss, R. A. Moravec and A. L. Niles, "Cell Viability Assays," in *Assay Guidance Manual,* Eli Lilly & Company and the National Centre for Advancing Translational Sciences, 2013, pp. 1-25.

[255] M. F. Wolf, K. P. Coleman and G. M. Lewerenz, "In Vitro Assessment of Cell and Tissue Compatibility," pp. 593-608.

[256] M. C. Vemuri, L. G. Chase and M. S. Rao, Mesenchymal Stem Cell Assays and Applications, Humana Press, 2011.

[257] Y. Cai, Titanium Dioxide Photocatalysis in Biomaterials Applications, Uppsala: Acta Universitatis Upsaliensis, 2013.

[258] K. Li, J. Yu, Y. Xie, L. Huang, X. Ye and X. Zheng, "Chemical stability and antimicrobial activity of plasma sprayed bioactive Ca2ZnSi2O7 coating," *Journal of Materials Science: Materials in Medicine,* vol. 22, pp. 2781-2789, 2011.

[259] M. Roy, G. A. Fielding, H. Beyenal, A. Bandyopadhyay and S. Bose, "Mechanical, In Vitro Antimicrobial and Biological Properties of Plasma Sprayed Silver-Doped Hydroxyapatite Coating," *ACS Applied Materials & Interfaces,* vol. 4, no. 3, pp. 1341-1349, 2012.

[260] P. A. Belanger, J. Beaudin and S. Roy, "High-throughput screening of microbial adaptation to environmental stress," *Journal of Microbiological Methods,* vol. 85, no. 2, pp. 92-97, 2011.

[261] J. P. Diaper, K. Tither and C. Edwards, "Rapid assessment of bacterial viability by flow-cytometry," *Applied Microbiology and Biotechnology,* vol. 38, no. 2, pp. 268-272, 1992.